FACTORS IN SALIVA CORRELATED WITH DENTAL CARIES[1]

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Previous studies by the author (1) of stimulated saliva of persons with active dental caries, on one hand, and free from caries, on the other, point to the conclusion that differences, valid in group averages, occur between the two groups in several respects. The caries-free group gave higher mean values for carbon dioxide capacity,[2] total calcium, inorganic phosphate, and the percentage of calcium removed after shaking saliva with a preparation of commercial tricalcium phosphate,[3] while the group with active caries yielded a higher aver-

[1] This investigation was conducted with the aid of a grant for dental research from the Carnegie Corporation. Presented at a meeting of the New York Section of the International Association for Dental Research, June 2, 1938.

[2] The carbon dioxide capacity is a measure of the neutralizing power of saliva against acids.

[3] The differences in the percentages of calcium and phosphorus removed when saliva is shaken with this preparation are probably the result of differences in the forms in which calcium and phosphate are present in the saliva of caries-free and active-caries groups. Since commercial tricalcium phosphate, employed in these experiments, is not uniform in composition (6, 7, 8), samples of a single preparation were used throughout these studies, including the present one. The Ca:P ratio of the preparation was 1.6. Hodge, LeFevre, and Bale (8) analyzed 2 samples of commercial tribasic calcium phosphate with Ca:P ratios of 1.7 and 1.66, and stated that they may be approximated by a mixture of 60 per cent $Ca_3(PO_4)_2$ and 40 per cent $CaHPO_4$. They found that roentgen ray diffraction patterns of these 2 samples showed lines of both tertiary and secondary calcium phosphates. Preparations acting on saliva like the tricalcium phosphate used in the present studies have been prepared by the author from calcium carbonate and phosphoric acid. Since commercial $CaCO_3$ is itself variable in composition, it is necessary to ascertain the proportion of H_3PO_4 and $CaCO_3$ which yields the desired preparation. The procedure is as follows: $CaCO_3$ (Mallinckrodt, Analytical Reagent), in amounts ranging at 2 grams intervals from 125 to 135 gms., was added to phosphoric acid solutions prepared by adding 130 grams of H_3PO_4, 85 per cent (Mallinckrodt, A. R.) to 680 cc. of water and heating. The $CaCO_3$ was added in small amounts (about 7 gms. at a time) with constant stirring. It generally required about 20 minutes for the addition of the total amount of $CaCO_3$. The mixtures were then heated for 5 minutes with frequent stirring, allowed to stand

age value for phosphorus removed after similar treatment. More recently, in a study of the same characteristics in stimulated saliva of Eskimos free from caries and with active caries, Karshan, Rosebury, and Waugh (2) found average differences between the two groups, similar in direction to those previously reported (1). A detailed review of other studies along these lines has been published recently (3).

In a continuation of the investigation of the relationship of saliva to dental caries, the series of cases for which paraffin-stimulated saliva was studied has been extended, and in addition unstimulated saliva, collected with minimum movement of the tongue, cheeks, lips, and jaw, has been analyzed. As in the previous study (1), the subjects were divided into 4 groups—caries-free, arrested caries, active caries, and miscellaneous.

Each individual in the caries-free group had a full set of teeth, free from cavities and fillings, as shown by thorough mouth examination and checked by radiograph. All doubtful cases, such as those having pits or fissures large enough to admit an explorer, were excluded from this group. In the arrested-caries group, no unfilled cavities were present; the fillings were small, few, occurred occlusally, and were prepared at least 5 years ago. The active-caries group included those who had active decay apparently of recent origin, associated with definite disintegration of enamel, accompanied by softened dentin. The number of cavities per person varied from a few to many; in subjects having only a few, proximal surfaces were involved. In these cases, teeth other than those with large cavities had areas of decalcification, usually at the gingival margin. Individuals having

overnight and the supernatant fluid decanted. The precipitate was washed 4 times with 1000 cc. portions of water, which was decanted after each washing. After the final decantation, additional water was removed by centrifugation and the precipitate was dried at 100° C. for 24 hours. The dried material was sifted through an 80 mesh sieve and kept in a desiccator containing $CaCl_2$. Five gram samples of these preparations and the commercial preparation of tricalcium phosphate were treated with 10 cc. portions of saliva as described under "methods" to determine the amount of $CaCO_3$ required with the stated amount of H_3PO_4 to give a product which removes from saliva the same amounts of calcium and phosphorus as are taken out by the commercial preparation. The quantity of $CaCO_3$ required usually fell within the range given above. The desired product can also be obtained by adding to a preparation made with an excess of $CaCO_3$, a definite amount (determined by trial) of a preparation made with insufficient $CaCO_3$.

large cavities, with firm, discolored dentin, were not included. The miscellaneous group included individuals who had cavities or fillings, but could not be placed in either the arrested- or active-caries groups. The age range of the subjects studied was between 10 and 41 years, 90 per cent of the cases being between the ages of 15 and 25 years. Two or 3 sets of analyses were performed in about 75 per cent of the subjects, usually at intervals of 1 week.

METHOD

Saliva was collected in test tubes in 2 portions, approximately 15 minutes apart, 1 to 3 hours after breakfast. The first portion (15 cc.) was used for determinations of calcium, inorganic phosphate, and protein, and of the fractions of these constituents removed from saliva on shaking with commercial tribasic calcium phosphate; the second portion (8 cc.) was used for the determination of the carbon dioxide capacity. Mercuric chloride (0.05 cc. of a 2 per cent solution) was added to the second portion during collection to prevent changes in the CO_2 capacity. To remove fractions of calcium, phosphorus and protein, 5 grams of the tri-calcium phosphate were added to 10 cc. of saliva, the mixture was shaken for 2 hours, centrifuged, and the supernatant fluid analyzed. In most instances it was necessary to centrifuge from 3 to 5 times before all suspended matter was removed. The analytical procedures were similar to those used previously (1). In the determination of the CO_2 capacity of stimulated saliva, 3 cc. were equilibrated with alveolar air, and 1 cc. taken for analysis. In the case of unstimulated saliva, 4 cc. were treated with alveolar air and 2 cc. taken for analysis. It should be noted that when 2 cc. instead of 1 cc. are used for the determination of CO_2 capacity by the method of Van Slyke and Neill (4), the pressure reading is applied in the regular way to the nomogram. The resulting CO_2 capacity is then divided by 2, and 1 volume per cent is subtracted from this result (5).

The *statistical* method employed for the interpretation of the results was the standard procedure of dividing the difference between the means of 2 groups (D), by the standard error (standard deviation) of the difference (σ D) to determine the probability that the observed difference was not due to chance.

RESULTS

Unstimulated saliva. The results for unstimulated saliva of individual cases are given in Tables I to IV. The statistical analysis of the data is presented in Table V. Higher mean values were found in the caries-free and arrested-caries groups than in the active-caries group for carbon dioxide capacity, total calcium, inorganic phosphate, and the per cent of calcium removed from saliva on shaking with a commercial preparation of tribasic calcium phosphate. The direction of these differences is the same as that reported previously (1) for stimulated saliva.

The similarity of the results of the caries-free and arrested-caries

groups is striking, and as shown in Table V, none of the differences between the means of these 2 groups are significant.

The differences between the means of the caries-free and active caries groups for carbon dioxide capacity, total calcium, inorganic

TABLE I

Analysis of unstimulated saliva of caries-free group

CASE NO.	NUMBER OF SETS OF ANALYSES	AGE	TOTAL Ca	INORGANIC P	CALCIUM REMOVED	PHOSPHORUS REMOVED	CO_2 CAPACITY cc./100 cc.	PROTEIN	
			Mg./100 cc.		Per cent			Mg./100 cc.	Per cent Removed
3	3	20	5.7	17.8	59	63	14.8	214	—
4	4	20	8.0	23.7	41	53	14.7	452	64
124	3	22	8.1	28.2	52	79	3.3	389	46
132	4	15	5.8	16.5	41	64	12.9	360	63
136	2	13	6.0	14.5	61	42	17.0	307	60
93	1	16	7.0	17.0	57	60	11.0	324	58
94	1	15	9.2	15.6	79	44	18.8	295	64
58	2	20	6.8	12.6	60	50	16.1	268	34
67	2	20	7.1	18.4	59	63	14.4	345	58
62	1	20	6.3	16.7	71	65	14.7	259	53
103	2	18	7.1	13.8	59	68	13.5	294	57
105	2	24	6.3	17.9	36	74	8.2	312	59
102	2	23	6.0	24.0	41	71	9.1	402	57
108	2	18	5.2	12.8	49	55	10.1	249	60
109	2	18	6.4	17.0	41	58	17.3	457	56
125	1	18	6.6	21.4	53	71	11.4	362	59
159	2	15	5.8	19.2	69	63	15.0	250	54
161	2	15	5.7	24.7	73	67	9.2	263	52
166	2	11	5.8	14.9	52	64	25.0	194	65
164	2	26	5.2	17.4	42	73	13.5	209	63
165	1	23	5.5	23.8	64	70	11.4	369	66
Mean.....		18.6	6.5	18.5	54	63	13.4	313	57
Standard Deviation.....			0.99	4.18	11.8	9.4	4.4	73.5	7.1
Standard Error of Mean.....			0.22	0.93	2.6	2.1	0.97	16.5	1.6

phosphate, and percentage of calcium removed on shaking with commercial tricalcium phosphate and carbon dioxide capacity were respectively 2.9, 2.9, 3.5, and 5.7 times the standard errors of the differences, indicating that the observed differences are unlikely to be due

to chance. The differences between the arrested-caries and active-caries groups for the same salivary characteristics are also such as to make it unlikely that they could be attributed to chance.

The miscellaneous group resembled the caries-free group in the mean values for calcium and inorganic phosphate, but gave values intermediate between the caries-free and active caries groups for calcium removed and CO_2 capacity.

TABLE II

Analysis of unstimulated saliva of arrested-caries group

CASE NO.	NUMBER OF SETS OF ANALYSES	AGE	TOTAL Ca	INORGANIC P	CALCIUM REMOVED	PHOSPHORUS REMOVED	CO_2 CAPACITY cc./100 cc.	PROTEIN	
			Mg./100 cc.		Per cent			Mg./100 cc.	Per cent Removed
61	3	20	6.8	19.3	44	64	12.1	367	62
84	2	20	7.4	17.2	71	49	17.5	342	62
82	2	20	5.6	19.1	19	75	5.2	464	72
107	2	27	6.7	21.0	44	64	12.8	389	44
104	1	18	5.6	22.2	60	66	9.5	375	58
113	2	18	6.5	15.8	64	55	15.0	285	55
114	2	18	7.7	17.6	58	67	9.5	310	53
26	3	20	5.6	15.1	40	52	16.2	—	—
28	3	19	6.5	19.3	27	35	18.4	—	—
151	3	22	7.2	26.1	69	70	11.4	282	58
19	3	20	5.7	18.2	65	54	20.3	—	—
5	2	20	5.5	16.9	27	63	13.0	269	48
162	1	31	6.9	14.4	62	70	7.0	218	42
Mean		21.0	6.4	18.5	50	60	12.9	330	55
Standard Deviation			0.73	3.02	16.9	10.4	4.3	67.8	8.7
Standard Error of Mean			0.22	0.87	4.9	3.0	1.24	22.6	2.9

Regarding the total protein content and the per cent of phosphorus and protein removed after shaking unstimulated saliva with tricalcium phosphate, although small differences were obtained between the various groups, it appears from the statistical analysis in Table V that the differences are without significance.

Stimulated Saliva. The results of the further study of stimulated saliva are presented in Table VI, which contains also the mean values

previously published (1) and the means of the combined first and second series. The results of the statistical analysis of the combined

TABLE III

Analysis of unstimulated saliva of active-caries group

CASE NO.	NUMBER OF SETS OF ANALYSES	AGE	TOTAL Ca	INORGANIC P	CALCIUM REMOVED	PHOSPHORUS REMOVED	CO_2 CAPACITY cc./100 cc.	PROTEIN	
			Mg./100 cc.		Per cent			Mg./100 cc.	Per cent Removed
1	1	13	5.9	14.8	44	59	6.0	—	—
2	1	15	—	—	—	—	8.5	—	—
8	1	20	4.9	11.1	—	77	1.0	—	—
60	2	20	7.2	20.8	42	77	6.5	394	67
64	2	20	5.4	13.5	34	59	13.8	244	54
83	3	20	7.5	23.7	25	77	4.8	387	60
106	2	17	4.3	11.3	43	56	14.4	177	45
112	2	17	6.9	14.0	45	67	14.5	320	68
122	3	10	4.2	13.8	−3*	72	5.8	268	49
130	2	17	6.9	13.9	34	63	11.9	—	—
131	2	14	4.6	13.9	50	37	22.4	—	—
133	2	12	4.8	15.6	−9	76	7.5	—	—
24	1	20	6.5	13.8	29	66	13.0	403	72
31	2	22	5.0	13.4	29	57	16.6	356	63
128	2	21	5.4	19.0	17	77	6.5	262	55
29	2	20	6.0	11.4	−11	65	8.6	330	67
11	1	20	5.5	15.2	16	59	8.0	386	43
119	3	16	4.9	11.3	15	63	9.7	245	56
145	3	20	5.5	13.2	21	70	6.0	419	69
80	3	14	5.3	11.8	−9	67	7.5	294	62
154	1	29	5.7	12.7	—	77	2.3	505	75
20	1	16	5.5	12.8	40	66	14.7	—	—
158	2	25	6.3	16.1	37	70	9.4	355	59
163	2	15	5.9	13.4	52	67	11.7	247	50
171	2	30	6.3	19.7	22	80	6.7	438	57
Mean		18.5	5.7	14.6	26	67	9.5	335	59
Standard Deviation			0.88	3.16	19.0	9.4	4.7	82.2	9.0
Standard Error of Mean			0.17	0.66	4.1	2.0	0.96	19.9	2.2

* The minus sign means that calcium was added to the saliva.

series are given in Table VII. The new data are in essential agreement with those previously published for the caries-free and active-caries groups. However, the miscellaneous group differed in several

respects in the 2 series, yielding in general, mean values in the combined series which tended to approach more closely values intermediate between the caries-free and active-caries groups.

TABLE IV

Analysis of unstimulated saliva of miscellaneous group

CASE NO.	NUMBER OF SETS OF ANALYSES	AGE	TOTAL Ca	INORGANIC P	CALCIUM REMOVED	PHOSPHORUS REMOVED	CO_2 CAPACITY cc./100 cc.	PROTEIN	
			Mg./100 cc.		Per cent			Mg./100 cc.	Per cent Removed
134	2	36	5.4	17.8	22	67	8.7	—	—
153	3	31	4.7	14.5	33	67	12.0	299	61
101	2	27	7.2	29.9	56	72	8.5	412	53
100	3	30	6.2	17.6	39	68	7.5	416	55
146	1	22	5.4	20.6	51	67	13.0	241	45
147	1	20	6.5	24.5	36	77	8.2	380	61
150	1	20	7.1	24.3	—	—	10.4	—	—
155	1	21	7.5	23.6	62	49	9.8	251	51
6	2	20	7.4	12.4	54	59	12.2	—	—
167	2	20	4.9	14.5	16	69	13.6	269	62
168	1	20	7.5	22.7	37	63	14.7	500	38
169	2	20	7.2	16.8	37	71	11.4	521	66
170	2	20	7.7	17.5	54	74	12.5	286	60
173	1	16	6.1	17.1	10	80	5.5	375	53
174	1	20	5.7	17.9	37	80	8.0	279	54
178	2	20	5.3	14.3	48	62	16.5	243	49
179	2	21	4.1	17.2	9	76	8.1	267	60
180	2	20	7.8	14.9	48	79	8.3	224	49
181	2	20	5.7	16.5	50	64	17.7	252	—
49	2	18	6.9	20.1	38	74	8.6	357	60
Mean		22.1	6.3	18.7	39	69	10.8	328	55
Standard Deviation			1.09	4.30	15.0	7.8	3.1	89.7	7.1
Standard Error of Mean			0.24	0.97	3.6	1.8	0.72	22.4	1.8

With the exception of the results for protein, the conclusions which can be drawn from the statistical analysis of the data in Table VII are, in general, similar to those previously reported by the author (1) for stimulated saliva. The significance of the differences is, however, increased considerably as a result of the larger number of cases studied.

TABLE V

Statistical analysis of data of unstimulated saliva of caries-free, arrested-caries and active-caries groups

	GROUP	GROUP MEAN	$\frac{D^*}{\sigma D}$	ODDS AGAINST THE CHANCE OCCURRENCE OF DIFFERENCE BETWEEN MEANS	GROUP	GROUP MEAN	$\frac{D}{\sigma D}$	ODDS AGAINST THE CHANCE OCCURRENCE OF DIFFERENCE BETWEEN MEANS	GROUP	GROUP MEAN	$\frac{D}{\sigma D}$	ODDS AGAINST THE CHANCE OCCURRENCE OF DIFFERENCE BETWEEN MEANS
Calcium, Mg./100 cc.	Caries-free	6.5	2.9	267 to 1	Arrested caries	6.4	2.6	106 to 1	Caries-free	6.5	0.3	<1 to 1
	Active caries	5.7			Active caries	5.7			Arrested caries	6.4		
Inorganic Phosphate, Mg./100 cc.	Caries-free	18.5	3.5	2149 to 1	Arrested caries	18.5	3.6	3142 to 1	Caries-free	18.5	0	<1 to 1
	Active caries	14.6			Active caries	14.6			Arrested caries	18.5		
Calcium Removed, Per cent	Caries-free	54	5.7	$>10^8$ to 1	Arrested caries	50	3.8	6915 to 1	Caries-free	54	0.7	1 to 1
	Active caries	26			Active caries	26			Arrested caries	50		
Phosphorus Removed, Per cent	Caries-free	63	1.3	4 to 1	Arrested caries	60	1.9	16 to 1	Caries-free	63	0.8	1 to 1
	Active caries	67			Active caries	67			Arrested caries	60		
Carbon Dioxide Capacity, Cc./100 cc.	Caries-free	13.4	2.9	267 to 1	Arrested caries	12.9	2.2	35 to 1	Caries-free	13.4	0.3	<1 to 1
	Active caries	9.5			Active caries	9.5			Arrested caries	12.9		
Protein, Mg./100 cc.	Caries-free	313	0.9	2 to 1	Arrested caries	330	0.2	<1 to 1	Caries-free	313	0.7	1 to 1
	Active caries	335			Active caries	335			Arrested caries	330		
Protein Removed, Per cent	Caries-free	57	0.7	1 to 1	Arrested caries	55	1.1	3 to 1	Caries-free	57	0.6	<1 to 1
	Active caries	59			Active caries	59			Arrested caries	55		

* Difference between two group means divided by the standard error of the difference.

As in the case of unstimulated saliva, higher mean values were found in the stimulated saliva of caries-free and arrested-caries groups than in that of the active-caries group for carbon dioxide capacity, total calcium, inorganic phosphate, and per cent of calcium removed from saliva on shaking with tricalcium phosphate. On the other hand, the per cent of phosphorus removed after treatment with tri-

TABLE VI

Mean values for stimulated saliva of caries-free and caries groups

TYPE OF CASE	NO. OF CASES	NO. OF SETS OF ANALYSES	AGE	TOTAL Ca	INORGANIC P	CALCIUM REMOVED	PHOSPHORUS REMOVED	CO_2 CAPACITY cc./100 cc.	PROTEIN	
				Mg./100 cc.		Per cent			Mg./100 cc.	Per cent Removed
Second Series—New Data										
Caries-free	11	20	19.0	5.8	13.5	65	24	29.3	239	53
Active-caries	14	42	18.5	5.0	11.6	27	47	19.4	289	58
Miscellaneous	18	33	22.1	6.0	12.5	52	30	28.4	308	53
First Series—Previously Published										
Caries-free	22	64	18.7	6.1	13.4	65	18	31.1	266	50
Arrested-caries	15	35	23.2	6.1	13.9	68	21	30.2	285	52
Active-caries	41	104	19.1	5.3	12.2	43	44	19.5	283	56
Miscellaneous	27	67	20.5	5.3	13.6	46	40	20.8	310	55
First and Second Series Combined										
Caries-free	33	84	18.8	6.0	13.4	65	20	30.5	258	51
Arrested-caries	15	35	23.2	6.1	13.9	68	21	30.2	285	52
Active-caries	55	146	18.9	5.2	12.1	39	45	19.5	284	56
Miscellaneous	45	100	21.2	5.6	13.2	48	36	23.9	309	54

calcium phosphate was less in the caries-free than in the other 2 groups.

The differences between the caries-free and active-caries groups for all the above salivary characteristics were such as to make it very unlikely that they could have arisen merely by chance (Table VII). Significant differences in the same salivary characteristics were likewise found between the arrested-caries and active-caries groups.

TABLE VII

Statistical analysis of data of stimulated saliva of caries-free, arrested-caries, and active-caries groups

	GROUP	GROUP MEAN	$\frac{D}{\sigma D}$	ODDS AGAINST THE CHANCE OCCURRENCE OF DIFFERENCE BETWEEN MEANS	GROUP	GROUP MEAN	$\frac{D}{\sigma D}$	ODDS AGAINST THE CHANCE OCCURRENCE OF DIFFERENCE BETWEEN MEANS	GROUP	GROUP MEAN	$\frac{D}{\sigma D}$	ODDS AGAINST THE CHANCE OCCURRENCE OF DIFFERENCE BETWEEN MEANS
Calcium, Mg./100 cc.	Caries-free	6.0	4.0	15770 to 1	Arrested caries	6.1	3.6	3142 to 1	Caries-free	6.0	0.4	<1 to 1
	Active caries	5.2			Active caries	5.2			Arrested caries	6.1		
Inorganic Phosphate, Mg./100 cc.	Caries-free	13.4	3.1	516 to 1	Arrested caries	13.9	3.5	2149 to 1	Caries-free	13.4	0.9	2 to 1
	Active caries	12.1			Active caries	12.1			Arrested caries	13.9		
Calcium Removed, Per cent	Caries-free	65	8.1	$>10^{11}$ to 1	Arrested caries	68	9.0	$>10^{11}$ to 1	Caries-free	65	1.4	5 to 1
	Active caries	39			Active caries	39			Arrested caries	68		
Phosphorus Removed, Per cent	Caries-free	20	7.5	$>10^{11}$ to 1	Arrested caries	21	4.9	$>10^{6}$ to 1	Caries-free	20	0.2	<1 to 1
	Active caries	45			Active caries	45			Arrested caries	21		
Carbon Dioxide Capacity, Cc./100 cc.	Caries-free	30.5	7.2	$>10^{11}$ to 1	Arrested caries	30.2	4.9	$>10^{6}$ to 1	Caries-free	30.5	0.1	<1 to 1
	Active caries	19.5			Active caries	19.5			Arrested caries	30.2		
Protein, Mg./100 cc.	Caries-free	258	2.4	60 to 1	Arrested caries	285	0.06	<1 to 1	Caries-free	258	1.8	13 to 1
	Active caries	284			Active caries	284			Arrested caries	285		
Protein Removed, Per cent	Caries-free	51	2.4	60 to 1	Arrested caries	52	2.1	27 to 1	Caries-free	51	0.4	<1 to 1
	Active caries	56			Active caries	56			Arrested caries	52		

No significant differences were found between the caries-free and arrested-caries groups.

The mean values for total protein and protein removed after shaking stimulated saliva with tricalcium phosphate were less in the caries-free than in the active-caries group, and the differences in each case were greater than are likely to be due to chance. However, the arrested-caries group did not differ from the active-caries group in the mean values for total protein, although the difference between the means of the 2 groups for the per cent of protein removed as a result of treatment with tricalcium phosphate is probably significant.

SUMMARY AND DISCUSSION

In both stimulated and unstimulated saliva, higher mean values were found in the caries-free than in the active-caries group for carbon dioxide capacity (a measure of the ability of saliva to neutralize acid), total calcium, inorganic phosphate, and the per cent of calcium removed from saliva on shaking with a commercial preparation of tricalcium phosphate. A lower mean value in the per cent of phosphorus removed on shaking with tricalcium phosphate was found in stimulated saliva of the caries-free than in the active-caries group. In all these respects the differences between the mean values are statistically significant.

In stimulated saliva lower mean values for total protein and for protein removed by shaking the saliva with tricalcium phosphate were found in the caries-free than in the active-caries group. The differences between the means in each instance were such as to make it unlikely that they could be attributed to chance.

The differences in unstimulated saliva between the caries-free and active-caries groups in the protein content and in the per cent of phosphorus removed on shaking the saliva with tricalcium phosphate, were very small and not significant.

The findings in both stimulated and unstimulated saliva for groups of individuals having arrested caries were, in general, similar to those for the caries-free group, and different from those of the active-caries group, while in most respects the miscellaneous group was either intermediate between the caries-free and active-caries groups or similar to the caries-free group.

The differences in the action of tricalcium phosphate on both stimulated and unstimulated saliva of caries-free and active-caries groups probably indicates that either all or part of the calcium and phosphorus in stimulated saliva, and the calcium in unstimulated saliva, exists in the caries-free group in forms which differ from those in the active-caries group.

The findings which have been discussed are related to dental caries in so far as they are concerned with the conditions governing the destructibility or solubility of enamel. It has been shown that the solubility of enamel depends largely on the concentration of calcium and phosphate ions in the surrounding medium. The content of these ions in saliva is probably always great enough to prevent solution of enamel within the range of hydrogen-ion concentration that occurs in saliva, provided that the saliva has free access to the enamel surface. This fact doubtlessly explains in part the absence of caries on the more exposed surfaces of enamel. If we assume that saliva has partial access to the more secluded regions in which dental caries occurs, it would follow that this protective mechanism is interfered with or broken down in such regions when a lesion develops, and the high hydrogen-ion concentration that results from local bacterial action is able to cause solution of enamel. The potency of this protective mechanism in saliva evidently varies in different individuals, being influenced by the prevailing level of acid-neutralizing substances (as indicated by the CO_2 capacity), and by the concentrations of calcium and inorganic phosphate or the forms in which they exist. The findings given indicate that the saliva of groups of persons with active caries differs significantly from that of groups of persons free from caries, or with arrested caries, in these characteristics. In other words, the findings indicate that the saliva of persons free from dental caries would protect enamel against solution by acids to a greater extent than would the saliva of persons with active decay.

The author acknowledges his indebtedness to Miss Mary Segall for technical assistance, and to Prof. Daniel E. Ziskin and other members of the Division of Oral Diagnosis, who conducted the dental examinations.

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DIETARY EFFECTS UPON THE ACID NEUTRALISING POWER OF THE SALIVA[1]

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It has been found that the ability of the saliva to neutralise acids can be influenced by a large variety of means. It can be increased by introducing into the mouth alcoholic liquors, acids and other irritating substances (1, 5, 8); and by either active or passive movement of the jaws (1, 4, 5, 6, 10, 13). Foods increase the acid neutralising power of the saliva in the following order of increasing magnitude of effect: hard boiled eggs, roast chicken, black bread, boiled potato, sour red apples and citrus fruits (3, 7). Injection of insulin was found to increase the potential alkalinity of the saliva of depancreatised dogs (9).

The acid neutralising power of the saliva was found to be higher in the afternoon than in the morning (11). Dieminger (2) found the alkalinity of the saliva to be highest during fasting and to fall after breakfast. At the usual time for lunch it increased strongly, and fell during the 2–3 hours following the meal. Van der Molen and Offringa (4) found that after a meal the alkalinity of the saliva was lowered, although immediately afterwards it was usually slightly increased, but returned to its original level within 2 hours of completion of the meal. Pickerill (7) found that an acid diet (fruit, vegetables and meat rendered acid with sauces) materially increased both the amount and alkalinity of the saliva. A high cereal diet was found (12) to lower the acid neutralising power of the saliva, while a high meat, egg, vegetable and fruit diet increased it. The acid neutralising power decreased after a lunch of sandwiches, but fell only slightly after a meal of meat, vegetables, bread, dessert and coffee (13).

[1] Presented in part at the 14th General Meeting of the International Association for Dental Research, Louisville, Ky., March 14–15, 1936.

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The work to be reported here was undertaken to investigate more fully the effects of dietary factors upon the ability of the saliva to neutralise acid. Early in the investigation it was found that there was no apparent relationship between the acid neutralising powers of basal and stimulated salivas. This is well shown in Table I, which compares the acid neutralising power of resting saliva with that of saliva from the same subjects after chewing various materials for 5

TABLE I

Effect of chewing various materials for five minutes upon acid neutralising power of the saliva

SUBJECT	CHEWING GUM		SPONGE RUBBER		PARAFFIN	
	Before	After	Before	After	Before	After
F	0.9	3.1	1.6	3.5	1.4	5.0
F	1.3	2.8	1.7	2.8	1.7	6.0
J			acid	1.9	acid	3.9
J					2.3	3.0
Ma	0.6	1.7	1.2	2.5	acid	1.2
Ma	1.1	1.5	1.4	3.4	0.4	3.4
Ma	1.4	1.3			0.6	4.4
Ma	1.5	1.6			1.3	4.1
Mo	1.6	2.1	1.8	3.4	1.5	3.5
Mo	2.5	1.6	2.4	3.3	1.7	3.9
Mo	2.7	3.4	3.0	3.5	2.5	4.1
Mo	3.1	4.3				
P					1.3	4.1
P					1.8	6.1
P					2.2	5.7
W	1.2	4.0	1.7	3.9	1.9	4.8
W	2.2	4.5	2.0	3.4	2.1	6.2
W	2.7	3.3	2.4	4.6	2.3	5.1

minutes (acid neutralising power is expressed as defined below). It will be noted that in general the effect of the chewing was roughly proportional to the resistance offered the jaws by the material in the mouth, as has been reported by McClelland (6). Further, the elevation of the acid neutralising power produced by chewing was quite inconstant, in agreement with the work of Fosdick (14).

In view of the foregoing results it was decided to use in our further work resting saliva obtained by allowing fluid to collect in the mouth and then ejecting it into a test tube.

The persons finally chosen as experimental subjects all had relatively rapid resting salivary flows so that a sample (about 4 c.c.) usually could be collected in 5 to 10 minutes. The subjects were all males, and with one exception (subject F) were within an age group of 18–24 years. Subject F was about 40 years of age.

Except where otherwise noted, collections were made every second day at five o'clock in the afternoon—approximately 4 hours after lunch. Two drops of a 0.4% solution of methyl red in 95% ethyl alcohol were added to 3 c.c. of saliva, and the mixture was titrated with 0.01N HCl to the first appearance of a distinct red color. The number of c.c. of acid used in this titration was taken as an index of the acid neutralising power of the saliva.[3]

Effect of diet. The subjects of the experiments were given rough outlines of diets containing high and low percentages of carbohydrate. A third diet consisted of a free choice of foods. The subjects kept

TABLE II

Effect of diet on acid neutralising power of the saliva

SUBJECT	DIET C			DIET N			DIET P		
	No. of Dets.	Average A.N.P.	σ	No. of Dets.	Average A.N.P.	σ	No. of Dets.	Average A.N.P.	σ
B	12	1.4	0.2	30	1.7	0.4	14	2.3	0.1
F				73	1.6	0.4	34	2.3	0.3
J	12	0.4	0.3	35	1.2	0.4	72	2.0	0.4
Ma	32	1.2	0.5	37	1.7	0.4	11	2.3	0.4
Mo	29	1.9	0.4	47	2.3	0.4	73	2.9	0.3
W	12	1.9	0.3	35	2.7	0.5	13	3.1	0.3

σ = standard deviation.

accurate records of their actual food intakes on the various diets, and it was possible to work out later from this data the average compositions with respect to carbohydrate, fat, and protein of the three diets used—high carbohydrate (C), normal diet (N), and low carbohydrate diet (P). The average compositions of the diets are shown below:

Diet	*%Protien*	*%Fat*	*%Carbohydrate*
P	40	35	25
N	25	20	55
C	18	15	67

[3] Throughout the remainder of this paper the acid neutralising power of the saliva will be abbreviated A.N.P., and will be expressed in terms of c.c. of 0.01N HCl used in the methyl red titration.

The general plan of the experiments was to place the subjects on the different diets for successive periods of 3 to 4 weeks. The results of this work are shown in Table II.

It will be seen from this table that there was possibly no significant difference between A.N.P. on diet C and that on diet N, and similarly there was no significant difference between the values obtained on diets N and P. However, there was a distinctly significant difference between the values obtained while the subjects were on diet C and those got while they were on diet P. This difference might arise by the liberation in the body of different metabolic end-products from the foods composing the 2 diets, or it may depend upon the presence of such accessory food factors as vitamins and minerals in larger amounts in one diet than in the other. One difference between diet P and diet C is that the consumption of 5 per cent vegetables on the former diet was much higher than on the latter. That this is an important factor is shown by our finding that the addition of spinach to diets previously free of leafy vegetables increased the average A.N.P. by 0.5 c.c., whether the diet was high carbohydrate or high protein in type. At present, it is as impossible to explain this specific effect of spinach as it is to assign a mechanism whereby the different diets produce their different results.

It could be objected to the preceding work that the results obtained may arise from direct effects of impacted food particles in the mouth rather than from systemic effects. Two types of experiment were carried out to test the validity of this objection. In the first of these, the subjects behaved exactly as they had before except that they brushed their teeth vigorously after every meal and rinsed the mouth thoroughly with water, it having been determined previously that such a procedure in no way affected A.N.P. when the sample was taken more than twenty minutes after the brushing.[4] This mechanical removal of food particles from the mouth in no way changed the dietary effects. However, tooth brushing is notoriously inefficient as a method of removing impacted food debris, and it therefore became necessary to devise another experiment to determine whether or not the dietary effects upon A.N.P. were strictly systemic in origin.

[4] See also Pickerill (7) and Carlson and McKinsty (8).

One of the subjects (B) was accustomed over a period of about 2 months to having a modified Lashley cannula (15) attached to the mouth of the right parotid duct. He was then placed on different diets as usual. Samples of mixed saliva were collected in the ordinary way, and immediately afterwards the Lashley cannula was put in place for collection of a sample of parotid saliva. The results of this work are shown in Table III.

It will be seen that the A.N.P. of the parotid saliva was always slightly lower than that of mixed saliva. This result is somewhat different from that of Werther (16), who found that in dogs the parotid saliva had the highest alkalinity. Perhaps we have here a species difference. Table III also shows that the A.N.P. of mixed and parotid salivas varied in the same way with diet. This result

TABLE III

Effect of diet on acid neutralising power of mixed and parotid salivas of subject B (each figure calculated from 14 consecutive tests)

DIET	MIXED SALIVA		PAROTID SALIVA	
	Average A.N.P.	σ	Average A.N.P.	σ
N	1.5	0.3	1.3	0.2
P	2.3	0.1	2.1	0.4

σ = standard deviation.

leads us to believe that different diets affect A.N.P. through some systemic means.

Effect of single meals. In this experiment the subjects were given various types of lunches while on different diets. Determinations of A.N.P. were made at regular intervals before and after the meal. Typical results are shown in *fig. 1*. The lines connecting the points just before and just after the lunch have no meaning, as we know nothing of the course of the changes in A.N.P. during the meal. Immediately after the meal, without regard to type, there is a marked increase in A.N.P., probably resulting from the masticatory effort associated with eating. This seems to disappear in about 15 minutes after completion of the meal.

From *fig. 1* we see that the lunch corresponding in composition to the general diet caused a change along the line of the general tendency

of the diet. A lunch of opposite composition to the diet produced a change first in the reverse direction to that produced by the diet, with a final return towards the general dietary level.

In some of the immediately preceding experiments, the carbohydrate lunch consisted of 100 grams of various pure carbohydrates with 100 c.c. of water. It was found that sucrose, fructose and glucose were about equally effective in their action, while corn starch

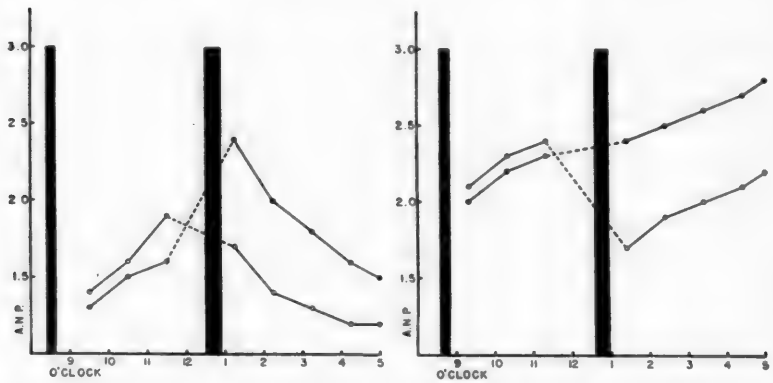

FIG. 1. Diurnal variations of acid neutralising power of saliva of subject W while on diet C (left) and on diet P (right). In each case open circles indicate values obtained when carbohydrate lunch was eaten, and solid circles those when protein lunch was taken. Vertical blocks represent breakfast and lunch.

apparently had no effect. The necessity that starch be hydrolysed before it can be absorbed may explain this difference.

SUMMARY

A diet low in carbohydrate and high in protein and 5 per cent vegetables increased the capacity of the saliva to neutralise acid. Conversely, a high carbohydrate diet lowered the acid neutralising power of the saliva. Such dietary effects upon the acid neutralising power of the saliva are believed to be produced by systemic means rather than by different types of fermentation of different food materials in the mouth. Single meals affect the acid neutralising power of the saliva in a manner depending upon their own character and upon that of the diet being taken.

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SOLUBILITY OF TOOTH ENAMEL IN SALIVA AT VARIOUS pH LEVELS[1]

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Various attempts have been made to study the effect of acids on decalcification of tooth enamel. Head (1) showed that saliva had a neutralizing power on acid phosphate solution in its decalcifying activity. This we know now is due to the buffering action of saliva. Others have found that saliva neutralizes lactic and other acids to such an extent that decalcification does not occur (2). Head's work was discounted by Pickerill on the ground that the acidity of the saliva and acid mixture was not determined and consequently the total effective acidity of the saliva was not known.

McClelland (3) weighed pieces of enamel and immersed them in buffered solutions for various lengths of time, finding that the pieces in solutions of pH 6.0 and below are decalcified at a rate proportional to the degree of acidity.

Dobbs (4) surmises that carbohydrates pass through dental plaques and are converted into acids by fermentation. If this is true, the pH of the dental plaque is more important than the pH of the saliva in tooth decalcification. Thus, the pH of a person's saliva might be high enough that no decalcification would occur and yet the pH of the plaque would be low enough to permit decalcification. Karshan and Rosebury (5) have studied the relationship of pH, calcium, and phosphorus to solubility of enamel with lactic, succinic, and malic acids on powdered enamel without addition of calcium and phosphorus. They stated that "the pH change at each time-level is strictly proportional to dissolved phosphorus at that level dissolved in the case of lactate buffers." However, this work was not performed in the presence of saliva. They stated that the calcium values are about

[1] Presented at the 17th General Meeting of the International Association for Dental Research, Cleveland, March 18–19, 1939 (*J. D. Res.*, **18**: 260, 1939).

twice the phosphorus concentrations in lactate and succinate buffers. This condition would be altered by the presence of calcium and phosphorus in saliva, as has been shown in our work.

Wendt and Clarke (6) determined the titration curve of phosphoric acid and calcium hydroxide at room temperature. They have decided that the final precipitate throughout the greater part of the precipitation range is tri-calcium phosphate. We can employ this data in the case of the calcium hydroxide—phosphoric acid mixtures to check the solubility product of tri-calcium phosphate, as has been done by Holt, La Mer, and Chown (7).

The solubility of tooth enamel has been roughly studied by various investigators, many of whom immersed whole teeth in acidic solutions. Rodriguez (8) and McIntosh, Jones, and Lazarus-Barlow (9) found no decalcification, while Hartzell and Larson (10) observed slight decalcification. Bunting and Palmerlee (11) and Friesell and Vogt (12) have exposed small areas of teeth to acidic solutions; the tests were qualitative, i.e., the appearance of the exposed area, as examined microscopically.

The activity of various acids in decalcification of enamel has been investigated by Thurlow and Bunzell (13) but they disregarded the pH of the solutions and the dissociation of acids, as well as solubility of calcium salt formed. McClelland (14) used buffer solutions of various pH levels in which to suspend their pieces of enamel and determined rate of dissolution by weighing before and after immersion.

Robb, Medes, McClendon, Graham, and Murphy (15) immersed apatite in "synthetic saliva" and concluded that no appreciable dissolution took place above pH 5.5. However, the solution was not circulated about the apatite so that little dissolution would be expected.

Attempts have been made to correlate susceptibility and immunity to dental caries with the calcium or the phosphate content of saliva. These investigators (16, 17, 18) have attempted to prove that dental caries is dependent on the chemical composition of the saliva, and have concluded that there is a correlation between high salivary calcium and immunity to caries. Other investigators (19, 20, 21) have been unable to check these observations. Since the ionic content of saliva varies at different times in the same individual, Clark

and Levine (20) conclude that it would be very difficult to make a correlation of this kind. Karshan, Krasnow, and Krejci (22) report slightly higher salivary calcium in immune patients, but they state that "the difference is not so great as some others have reported," i.e., too slight to be significant.

Those investigators who have attempted to correlate salivary phosphate with dental caries, disagree widely in their results. Hawkins (23) found the content to be lower for caries free individuals than immunes; Karshan, Krasnow, and Krejci (22) have found the phosphate concentration to be higher in immunes, while Entin and Geikin (24) and Horton, Marrack, and Price (18) could find no correlation. Hubbell and Bunting (25) have found no correlation between calcium and phosphorus content of saliva and occurrence of caries. They present data concerning the variation of calcium and phosphorus of saliva with the rate of secretion. No satisfactory correlation was obtained, although they believe that there is a tendency for the caries-free patients to secrete more saliva than susceptible persons.

Hubbell (26) in very complete analyses of children's saliva found that the titratable alkalinity of the saliva of caries-free children was about 24 per cent higher and the carbon dioxide capacity was about 44 per cent higher than in the cases of children who had active caries. In other respects, analyses of saliva (total solids, ash, calcium, phosphorus, chloride, diastatic activity, and pH) showed no consistent difference between the two groups.

The most common fault in experimentation in the study of the action of acids on enamel has been the neglect in maintaining a constant hydrogen ion concentration. However, by allowing a continuous stream of acid to flow over the enamel, and by decreasing the size of the exposed surface, so that the products of the dissolution will not retard the progress of the reaction (by being present in relatively small quantities), this could be eliminated. Enright, Friesell, and Trescher (27) have used the above means in maintaining a constant hydrogen ion concentration, and have found that all the simple lactate and citrate buffer solutions from pH 4.0 to pH 8.0 etched enamel, especially during longer periods of immersion. When these buffers had been saturated with tri-calcium phosphate, only those buffers with a pH more acid than 5.0 etched the enamel. Theoretically,

even the more acid buffers should have caused no etching, if sufficient calcium and phosphate ions were present.

Forbes (28), in theorizing on the etiology of dental caries, suggests that the ionization of tri-calcium phosphate is affected by changing the pH.

Benedict and Kanthak (29) shook powdered enamel with buffers of various pH levels for short periods. However, these periods were too short for equilibrium to be reached, hence the amount of dissolution is too low. Their theoretical curve does not fit our experimental data since they assumed that the solubility product of tri-calcium phosphate was 10^{-27}. According to experimental data obtained by Holt, La Mer, and Chown (7) and that contained in this paper, the solubility product either is not constant or it varies due to an indeterminant calcium ion or phosphate ion concentration. Holt, La Mer, and Chown (7) have studied the titration curve of calcium hydroxide-phosphoric acid mixtures after ten days of continuous shaking at 38°. They found that the solubility product of tri-calcium phosphate is not constant, but found that a progressive decrease in the solubility product occurred as more calcium hydroxide was added to the mixture, due to a decrease in primary phosphate. Presence of foreign salts tended to increase the solubility product of the calcium phosphate. The effect of the inorganic salts of blood serum at approximately neutral conditions gave a solubility product of the order of 6×10^{-28} at 38°C. The solubility product in horse serum was about 1×10^{-26} at 38°C.

PROCEDURE

Various amounts of lactic acid (up to 8 drops of concentrated acid) were added to 20 cc. portions of saliva (stimulated saliva from a group of persons was well mixed before withdrawal of the portions). These portions were placed in pyrex tubes containing approximately 0.1 gr. of powdered tooth enamel[2] (slightly larger amounts of enamel were added when much lactic acid was present). Two drops of toluene were added to each tube to preserve the contents. The tubes were sealed and then placed in a thermostat at 38°C., where they were shaken for 12 to 20 days, in order that equilibrium be reached. After this period of shaking, the pH of the contents were taken (at room temperature) with a Coleman potentiometer (frequently checked with standard buffers); then the mixture was

[2] Ground enamel from many teeth, whose particles passed through a 325 mesh screen was used. According to Kanthak (31) there is no apparent relation between age and state of teeth in the solubility of the enamel.

centrifuged and samples were pipetted from the supernatant liquid for phosphorus, calcium, and lactic acid analyses. For phosphate determination, the Fiske and Subbarow (30) method was used. Some readings were taken on a comparison colorimeter, others on a photelometer. For calcium determination, the Clark-Collip (32) modification of the Kramer-Tisdall method was used. A Van Slyke manometric apparatus was used for the lactic acid analyses.

DATA

The results of the analyses of the saliva-enamel-lactic acid mixtures are presented in graphic form. As has been stated above, the contents of each tube, after having the pH taken, was subjected to calcium, phosphorus, and lactic acid analyses. *Fig. 1* shows how the calcium content of the mixtures varies with the pH. The variation of phosphorus concentration with pH is presented in *fig. 2*.

Values for the calcium and phosphorus concentrations were then read from these graphs and these concentrations were used in the calculation of the solubility product. (The interpolated values for the concentration tend to minimize the experimental errors in the analyses.) The variation of the solubility product with pH is shown in *fig. 3*. The concentration of lactic acid necessary to attain the required pH levels is presented in *fig. 4*.

DISCUSSION

Nernst has shown that the product of the molar concentrations of the ions of an insoluble salt is a constant.

Since in dissociation

$$Ca_3(PO_4)_2 \rightleftharpoons 3Ca^{++} + 2PO_4^{=}$$

the solubility product is expressed

$$(Ca^{++})^3 \times (PO_4^{=})^2 = K_{S.P.}$$

If the solubility product of tooth enamel were known, theoretically we should be able to tell when decalcification would occur. If it were possible to foresee carious conditions, perhaps preventive methods would alleviate them. We should be able, through knowledge of the effect of pH on enamel, to determine the acid concentration necessary to lower the pH to a point where decalcification would occur. This amount would necessarily differ in various individuals, due to the difference in buffering power and differences of calcium and phosphorus concentrations in the decalcifying medium (i.e., plaque material, saliva, etc.).

Whether it is possible or not to get a constant value for the solubility product, we will still be able to determine when the decalcification of the enamel would occur, by determining at what pH the saliva would not contain excess calcium and phosphate ions to fulfill the solubility product requirements, i.e., when the protective action of the saliva would be lost, the protection being due to the fact that the saliva is supersaturated in regard to calcium and phosphate ions; decalcification would not occur until these were utilized, due to the lowering of the pH. Hence, we can determine the solubility product of tri-calcium phosphate if we know the ion concentrations, provided that sufficient concentrations of ions are present to yield the tertiary salt, which would be the case in this experimentation.

It is clear, from a consideration of the ionization of tri-calcium phosphate, that the rate of dissolution is dependent on (a) the hydrogen ion concentration (the lower the pH, the more rapid the dissolution); (b) the product of the concentration of calcium and of phosphate ions (the greater the product, the slower the dissolution). In this case, the above facts allow us to work in slightly acid solutions, where the phosphate ion concentration is small in respect to the total phosphates present.

By analysing the supernatant liquid in the above mixtures, the concentrations of calcium ion are determined directly. The total phosphate is found by analysis and from the knowledge of the relation between the pH and the fraction of the total phosphate ionized as $PO_4^{=}$,[3] the actual phosphate ion concentration can be determined. These concentrations (calcium ion and phosphate ion) were used in computing the solubility product at various pH levels.

In the case of the enamel-saliva-lactic acid mixtures it was found that the titration curve resembled that of Holt, La Mer, and Chown (7). Thus it appears that the enamel must have a composition resembling that of tri-calcium phosphate. This has been ascertained by numerous investigators by direct analysis of the tooth enamel.

Upon comparison of the graphs of the calcium concentration against pH (*fig. 1*), and the phosphorus concentration against pH (*fig. 2*), the similarity of the general shapes of these graphs will be

[3] Found in tabulated form in Holt, La Mer, and Chown's paper (7).

noted. At the pH level of the neutral point (pH = 7), the solubility is relatively slight. As the pH is decreased, the concentrations of the

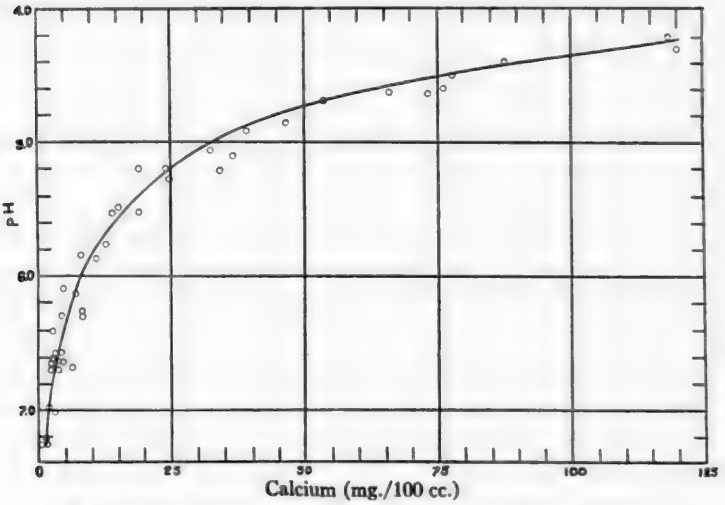

FIG. 1. Solubility of enamel in saliva at various pH levels—Calcium

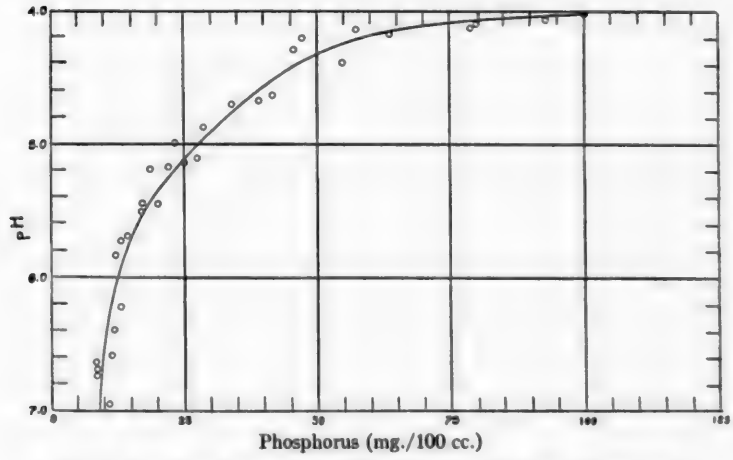

FIG. 2. Solubility of enamel in saliva at various pH levels—Phosphorus

ions increase slowly at first and later very rapidly, so that at a pH of 4 the concentrations are very large, indicating that if such acidic

conditions were existent in the mouth, rapid decalcification would be expected. The experimental evidence does not follow the theoretical concentrations of calcium and phosphate ions according to the mass action laws as shown in the graph of Solubility Product against pH (*fig. 3*). A variety of factors must be considered in explanation of our data.

The use of the term solubility product in the case of tooth enamel in saliva is probably a misnomer. For this reason, the term "apparent solubility product" will be used, to indicate the normal ionic product for tri-calcium phosphate. In this specialized case, however, it is impossible to accurately determine the solubility product for enamel. It will be noted that when the apparent solubility product is plotted against the pH, the values for the ionic product are relatively constant below a pH of 6.2, but that above this pH the value (*fig. 4*) rapidly increases. In order to get a constant for the solubility product for a compound, it must be pure and it must have a definite chemical formula. Tooth enamel is not pure tri-calcium phosphate (although its content of this compound is very high), so that a constant would not be expected when one computes the solubility product, when using the ionic expression for the solubility product of tri-calcium phosphate. Since concentrations of ions have been used instead of the activities of these ions, the exact solubility product would not be ascertained.

When one adds acid to a tri-calcium phosphate solution, the salt changes partially to secondary calcium phosphate, and perhaps primary calcium phosphate; however, the solubility products of these compounds are larger than that of tri-calcium phosphate, so that if sufficient ions are present to satisfy the solubility product of tri-calcium phosphate, then no precipitation of the other salts should occur. The phosphate ion concentration of the saliva is undoubtedly depressed due to the formation of the other salts. Values for this change have been referred to previously in this paper. This would be true only in the case of pure solutions of the salts. In the complex biological system, saliva, these are no doubt in error. The phosphate ion concentration might also be depressed by adsorption of the ion on mucin and other protein material in the mouth.

The calcium concentration is undoubtedly not entirely ionized if the nature of calcium in saliva is analagous to its form in blood

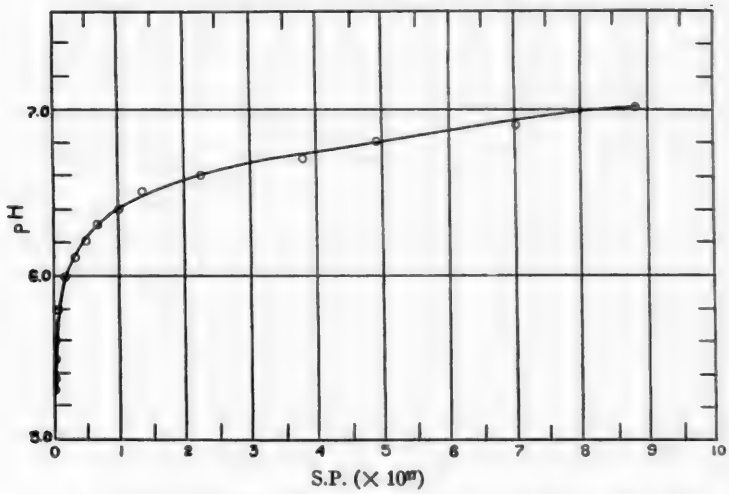

FIG. 3. Apparent solubility product of enamel in saliva at various pH levels

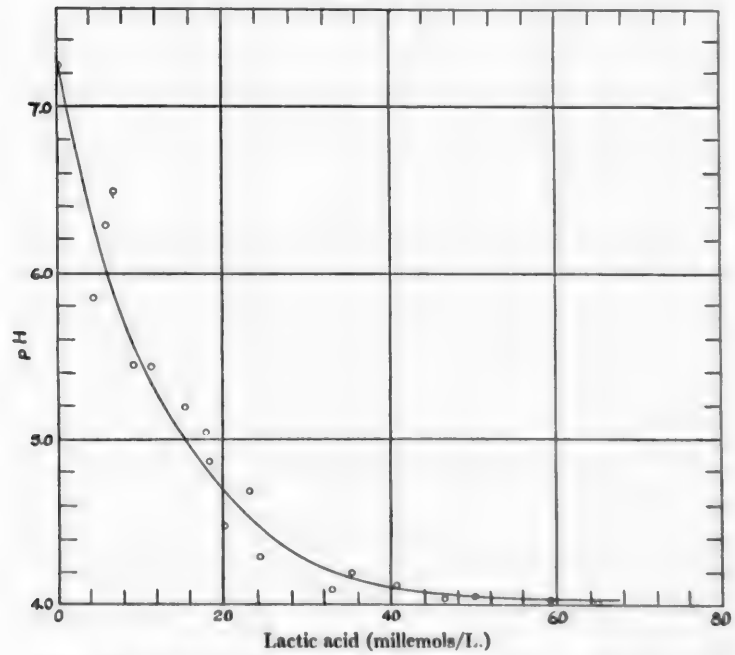

FIG. 4. pH level attained by addition of lactic acid to saliva—enamel mixtures

serum, where a portion of the calcium is not ionized. Hence, in the case of salivary calcium, a depression of the calcium ion concentration would lower the protective action due to the supersaturation of saliva with this ion. Also, perhaps a portion of the phosphate may be present in unionized form, such as phosphoproteins. This might be determined along with the ionized phosphates, but would play no part in the solubility product. In biological systems, such as in the case of saliva, the calcium and phosphate concentrations cannot be arrived at exactly, since their concentrations are depressed due to the influence of various non-ionized complexes.

Greenwald (33) studied the dissociation of calcium salts. The apparent solubility product for tri-calcium phosphate was determined in the presence of a number of organic anions. It was found that many of these so greatly increased the apparent solubility products as to indicate that their calcium salts were only slightly dissociated. The apparent solubility product for tri-calcium phosphate in the presence of calcium lactate at pH 7.30 was determined to be 17.2×10^{-30}. Calcium phosphate was found to accelerate the decomposition of both calcium glycerophosphates. Other anions caused greater variations. For instance, a change from a concentration of 1 millimol malate to 1 millimol fumarate changed the apparent solubility product from 20.8×10^{-30} to 3.5×10^{-30}, and the change from fumarate to maleate increased this to approximately 7.4×10^{-27}. Many other ions present in the saliva, such as potassium, chloride, sodium, ammonium, etc., would also affect the value of the solubility product.

Logan and Taylor (34) have investigated the action of acid on bone ash and have found that the calcium carbonate dissolved much more rapidly than the tricalcium phosphate. The carbonates in tooth enamel should also dissolve more rapidly, since this is an analogous case.

The uptake of phosphate ions in carbohydrate metabolism, according to the scheme presented by Fosdick and Hansen (35) would be an important factor in shifting the ionic equilibrium in the mouth; this factor would not interfere with the results of this experiment, since the saliva was preserved with toluene, hence none of the degradation products would be formed.

Most previous investigators of enamel decalcification have used whole teeth, presuming that immersion in a solution of known pH

would resemble mouth conditions. In this study it was deemed advisable to make the conditions as rigorous as possible, i.e., the use of small particles and the continuous shaking at body temperature, to attempt to find the maximum amount of decalcification that can take place at various pH's.

It has been ascertained (Fosdick and Campaigne (36)) that the pH averaged 6.4 in the case of non-carious areas and 5.9 in carious areas of the mouth. Stephan (37) by a suitable micro-technique tested the pH of 211 plaques. The average pH was 5.9, but the distribution was fairly even from pH 5.0 to pH 6.8. Debris from 38 tooth cavities was tested for hydrogen-ion concentration and was found to vary from pH 4.2 to pH 6.2. The average was pH 5.2. This was 0.7 pH below that of the plaques. Starr (38) found that the range of pH in 610 specimens of human saliva from 228 healthy patients to be pH 5.75–7.05, while 86 per cent of the values fell in a narrower range, pH 6.35–6.85. Brawley (39) reports in his study of the relationship of the pH of normal resting saliva to dental caries, that a definite relationship expressed itself as (a) low pH ranges, high caries indices; (b) high pH ranges, low caries indices. However, other investigators do not reach these conclusions.

Karshan, Krasnow, and Krejci (22) have reported that the average composition of stimulated susceptible saliva is as follows: Calcium, 5.3 ± 1.1 mg/100 cc.; phosphorus, 11.6 ± 1.6 mg/100 cc. Upon observation of the accompanying graphs, it can be seen that at pH 6.35, 5.3 mg. calcium/100 cc. are in solution and at pH 6.25, 11.6 mg. phosphorus/100 cc. are in solution. Hence, below a pH of 6.25 the saliva is unsaturated with regard to calcium and phosphorus, if the saliva had the above composition, so that decalcification of the teeth can occur. However, the pH beneath the plaques of the teeth are undoubtedly less than this value. The lower the pH, the more rapid should be the decalcification.

In order to determine the "critical pH," the pH that the salivary calcium and phosphorus satisfy exactly the solubility product requirements, (below this pH decalcification can occur) it was found necessary to plot the $\frac{\text{S.P.}}{\text{F}^2}$ against the pH. Since $(\text{Ca}^{++})^3 \times (\text{P} \times \text{F})^2 = \text{S.P.}$, where "$\text{Ca}^{++}$" and "P" represent the appropriate concentrations in saliva, "F", the fractional portion of the phosphorus ionized

as phosphate ion, and S.P., the solubility product of tricalcium phosphate, then,

$$(Ca^{++})^3 \times (P)^2 \times F^2 = S.P. \text{ or } (Ca^{++})^3 \times (P)^2 = \frac{S.P.}{F^2}$$

The accompanying graph (*fig. 5*) shows the relationship of the factor $\frac{S.P.}{F^2}$ and the pH.

If a sample of saliva is analysed for calcium and phosphorus, the product $(Ca^{++})^3 \times (P)^2$ can be determined. This product is equal

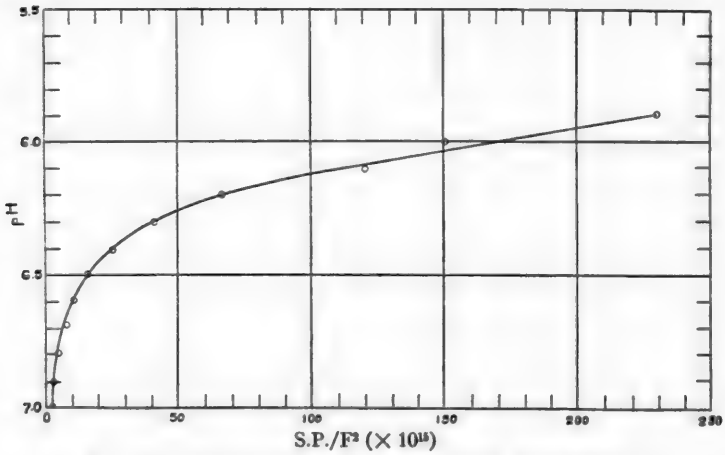

FIG. 5. Determination of "critical pH" (enamel decalcification)

to the factor $\frac{S.P.}{F^2}$. By interpolation from the graph, the pH at which the solubility product satisfies the requirement (numerical value for S.P.) is found. Below this "critical pH" decalcification can occur, since the solution will then be undersaturated in respect to calcium and phosphorus ions. At and above this pH, no decalcification can occur, since the solution is supersaturated in regard to these ions. For example, if a sample of saliva had a composition: calcium, 6.7 mg./100 cc., and phosphorus, 11.8 mg./100 cc.; the "critical pH" will be determined as 6.2. If the saliva had the composition: calcium, 4.0 mg./100 cc., phosphorus, 11.7 mg./100 cc.; the "critical pH" would be 6.5. It will be noted that the phosphorus concentration

is the same in these examples, while the calcium concentration varies. When the ionic concentrations are increased, the "critical pH" will be lower than when the ionic concentrations are decreased. This is in accord with theoretical speculation. The lactic acid concentration necessary to bring the mixture to a pH of 6.3 is 12 millimols lactic acid/1000 cc. (from analytical data). This is equivalent to a 0.024 molar lactic acid solution. It is plausible that such a solution could be formed in or beneath the dental plaques, or in carious areas.

SUMMARY

The calcium and phosphorus contents of saliva-tooth enamel-lactic acid mixtures at various pH levels were determined. The apparent solubility product was calculated at various pH levels and was found to vary over wide limits. The factors causing this variation were discussed. A method was suggested for the determination of the critical pH at which enamel will dissolve in saliva. This was correlated with the pH in isolated areas of the mouth and in carious lesions of the teeth.

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HUMAN SALIVA

VII. A Study of Rate of Flow of Resting Saliva[1]

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INTRODUCTION

Most investigators of mixed human saliva, in collecting samples for analysis, have shown a preference for either activated or resting saliva[2] (for reviews see: 3, 5, 18, 6, 26, 27). Some have stated definitely the reason for their choice, others have not been concerned with the difference between R.S. and A.S. as long as they have been able to secure samples large enough to perform the desired chemical analysis.

In early studies of human saliva only occasional interest was shown in variations of rate of flow and their effect on the composition of either R.S. or A.S. in spite of the fact pointed out by Pickerill (23, 24) that "The mere analysis of a sample of saliva in a test-tube without knowing anything as to conditions and rate of secretion is utterly valueless." The first detailed records for rate of flow of mixed human R.S. were reported by Becks (2) and White and Bunting (30); and for A.S. by Brown and Klotz (10, 11) and White and Bunting. Brown and Klotz recently demonstrated typical differences in composition of A.S. by classifying individuals according to the amount of saliva produced by paraffin activation. The records contained in these previous reports indicate the necessity of considering the rate of flow in an interpretation of analytical results. This is realized even more when the changes in salivary composition brought about by activation are considered. Clark and Levine (12), Forbes (13), Jonsgar (16),

[1] Conducted under grants made by the California State Dental Association, California Academy of Periodontology, Research Board of the University of California, and the Good Teeth Council for Children Inc.

[2] The symbols R.S. and A.S. will be used to designate resting and activated saliva.

Krasnow (17), Wach (25), and White and Bunting (30) showed that activation not only provides a much larger amount of saliva for analysis but also alters the composition typically. These observations confirm earlier conclusions, drawn from animal experiments, that alteration of the rate of flow has definite effects upon composition (Pavlow (20, 21, 22), Heidenhain (14), and Ludwig (19), among others).

In view of these findings the question of which type of sample should be used in routine salivary examinations has become a major problem. In all experimental and clinical work standard procedures must be established for preparation of patient, collection of salivary sample, and analytical procedure in order that a comparison may be made not only between findings of different investigators but also between results obtained from different patients and from the same patient on different days. Environmental and seasonal variations, the character of the individual's general metabolism, physical height and weight, the myriad of psychic and nervous impulses, and the diseased conditions of the organism, may influence human saliva to such an extent that it would be very difficult to arrive at definite standards for rate of flow and composition.

In addition to these factors it must be remembered that mixed human saliva is the secretory product of large and small glands distributed over the entire oral cavity. The secretions of these glands are governed by efferent cranial nerves (lingualis and chorda tympani for the sublingual and submaxillary glands; glossopharyngeal for the parotid gland) and branches of the sympathetic system. The saliva is changed in its composition according to the stimulation of the individual glands by specific nerve fibers which according to Pavlov and Heidenhain are divided into three groups: a. Secretory fibers, which control the secretion of water and salts; b. Trophic fibers, which determine the organic elements of the saliva; c. Anabolic fibers, which inhibit the secretory function and control the restitution of the gland. It is easily conceivable that it will be difficult to control exactly the impulses reaching these groups of nerve fibers in human beings. However, by the study of a large number of persons under controlled environmental and experimental conditions one can determine ranges of rate of flow and of salivary composition for the majority of persons with average impulses.

The great differences in the results of analyses of mineral composition of mixed human saliva reported in former publications (5, 6) prompted a more detailed study of variations in the rate of flow of R.S. and A.S. and an attempt to find answers to the following questions.[3] 1. Does the rate of flow of R.S. of a single individual fluctuate considerably over a period of months or years and is the mineral composition affected thereby? 2. Does the rate of flow of R.S. and A.S. fluctuate considerably on different days over short periods of time (successive days)? 3. Does the rate of flow of R.S. and A.S. fluctuate to a marked extent during one or more hours of continuous collection? (The time of collection varies with each investigator). 4. Which type of saliva, resting or activated, fluctuates more in a given time unit with regard to rate of flow and composition? 5. Do differences in rate of flow, not recorded by most investigators, explain the differences in the results reported for mineral composition? 6. Does a great fluctuation of rate of flow make it impossible to study the effect of medication, of changed dietary regime, or of other endogenous or exogenous factors on salivary composition over an extended period of time? 7. Is saliva, under standardized resting conditions, a secretory or excretory product or both? 8. Do mg. per cent calculations of salivary composition provide an adequate measure which can be used for comparative purposes in the case of a secretory product as for instance saliva? 9. Can the conclusions drawn by former investigators concerning a correlation or a lack of correlation between salivary calcium and phosphorus composition and dental caries be accepted if results are expressed in mg. per cent?

Answers to the above questions should make it possible to eliminate untenable conclusions derived from inadequately investigated material and should lead to the establishment of standards for rate of flow and composition of R.S. and A.S.

FLUCTUATIONS OF RATE OF FLOW OF RESTING SALIVA ON DIFFERENT DAYS DURING A TWO YEAR PERIOD

The fact that R.S. has been used in the past for analysis of mineral composition and the great variations in results were obtained made it necessary to investigate whether the rate of flow of R.S. is more or

[3] The first three of these problems are considered in part in this publication, the others will be taken up subsequently.

less constant on different days or whether great fluctuations must be expected. For this purpose data were collected from 40 individuals. The examinations extended over a period of from 1 to 2 years and sa-

TABLE I

*Rate of flow of resting saliva on different days**

GROUP I				GROUP II			
No.	A	B	C	No.	A	B	C
	Range cc./hr.	Fluctuation			Range cc./hr.	Fluctuation	
		cc./hr.	per cent			cc./hr.	per cent
1	15.0–17.0	2.0	13.3	21	20.0– 25.2	5.2	26.0
2	13.6–16.1	2.5	18.4	22	25.9– 34.1	8.2	31.7
3	13.1–15.8	2.7	20.6	23	31.3– 42.5	11.2	35.8
4	13.4–16.4	3.0	22.4	24	30.0– 42.9	12.9	43.0
5	16.5–20.3	3.8	23.0	25	21.7– 32.8	11.1	51.2
6	12.0–15.0	3.0	25.0	26	31.5– 48.3	16.8	53.3
7	16.1–21.1	5.0	31.0	27	22.3– 34.5	12.2	54.7
8	8.3–12.0	3.7	44.6	28	26.7– 41.5	14.8	55.4
9	7.2–10.9	3.7	51.4	29	25.2– 40.7	15.5	61.5
10	8.8–13.8	5.0	56.8	30	27.8– 47.1	19.3	69.4
11	12.8–20.8	8.0	62.5	31	23.4– 40.0	16.6	70.9
12	19.0–31.5	12.5	65.8	32	25.5– 45.0	19.5	76.5
13	15.0–25.6	10.6	70.7	33	30.0– 57.0	27.0	90.0
14	8.4–15.0	6.6	78.6	34	23.7– 46.0	22.3	94.1
15	6.0–10.8	4.8	80.0	35	41.0– 80.0	39.0	95.1
16	7.9–14.3	6.4	81.0	36	35.0– 73.3	38.3	109.4
17	5.7–10.9	5.2	91.2	37	31.2– 68.6	37.4	119.9
18	2.5– 4.8	2.3	92.0	38	28.0– 63.0	35.0	125.0
19	12.0–24.8	12.8	106.7	39	43.9–111.0	67.1	152.8
20	3.1– 7.7	4.6	148.4	40	26.4– 90.0	63.6	240.9
Range		2.0–12.8	13.3–148.4	Range		5.2–67.1	26.0–240.9
Av.	13.4	5.4	59.2	Av.	39.6	24.6	82.8

* From an average of 5 samples each, taken at intervals over a period of one to two years.

liva was collected 4, 5 or 6 times from each individual. The method of collection was the same as previously reported (27, 26, 6, 5).[4]

[4] Measurement of the amount collected is possible only if slender accurately calibrated vessels are used. The most satisfactory type is the conical 15 cc. pyrex centrifuge tube, calibrated to 0.1 cc. by the Pyrex company, with marks etched on a red stripe on one side of the glass.

It was noted at the beginning of these investigations that certain individuals had an extremely low rate of flow of R.S. which showed little fluctuation when taken after varying intervals; others with an originally high rate of flow showed a surprisingly great variation from day to day or month to month. For this reason the 40 individuals mentioned above were divided arbitrarily into Groups I and II. In Group I were listed 20 individuals with rates of flow up to 20 cc./hr. and in Group II, 20 others with higher rates of flow. Borderline cases which change from Group I to Group II were largely omitted in this study. The age[5] varied in Group I from 9 to 40 years and in Group II from 9 to 48. The sex[5] distribution in Group I was 10 males and 10 females; and in Group II 15 males and 5 females. The individual ranges of rate of flow are given in Table I (Columns A). From these were calculated the cc./hr. (Columns B) and the percentage fluctuations (Columns C). The average rate of flow of the individuals in Group I was 13.4 cc./hr. (Table I, Columns A, bottom row), and in Group II 39.6 cc./hr.

It is of interest to note that the average cc./hr. fluctuations (Table I, bottom row) are definitely lower in Group I than in Group II (5.4 as against 24.6 cc./hr.); and the same is found for the average percentage fluctuations (59.2 per cent as against 82.8 per cent). Therefore, the *lower the rate of flow of R.S. (Group I) the less fluctuation occurs over a period of 1 to 2 years; and the higher the rate of flow (Group II) the greater the fluctuation.*

The wide fluctuations in Group II may be normal variations or expressions of greater response to exogenous or endogenous factors. Seasonal variations and changed environmental conditions also may have a decided effect.

Our knowledge of the biological importance of mixed resting human saliva is very meager at the present time and it is impossible to state whether a low or high rate of flow, smaller or greater fluctuation, and finally higher or lower mineral concentration, is more desirable. We do not even know whether the extreme findings in Group II, Table I, represent normal values of R.S. It is conceivable that some of them

[5] The age and sex of all individuals reported in this paper were recorded. The data have been omitted because of limitations of space.

resulted from stimulation by certain exogenous factors unknown to the investigator. This again emphasizes the need for the establishment of standard ranges for rate of flow of R.S.

FLUCTUATIONS OF RATE OF FLOW OF RESTING SALIVA ON FIVE SUCCESSIVE DAYS

In order to determine whether the long intervals between the collections reported above were responsible for the variations, samples

TABLE II

Rate of flow of resting saliva on 5 successive days

GROUP I				GROUP II			
No.	A	B	C	No.	A	B	C
	Range cc./hr.	Fluctuation			Range cc./hr.	Fluctuation	
		cc./hr.	per cent			cc./hr.	per cent
1	11.6–12.4	0.8	6.9	11	20.4–23.2	2.8	13.7
2	17.6–20.0	2.4	13.6	12	32.0–38.4	6.4	20.0
3	15.2–17.6	2.4	15.8	13	52.8–63.6	10.8	20.4
4	16.0–20.4	4.4	27.5	14	35.6–52.4	16.8	47.2
5	12.0–16.0	4.0	33.3	15	60.8–90.4	29.6	48.7
6	14.8–20.0	5.2	35.1	16	40.0–62.0	22.0	55.0
7	15.2–22.4	7.2	47.4	17	26.0–44.0	18.0	69.2
8	6.4–10.8	4.4	68.8	18	22.0–42.4	20.4	92.7
9	6.0–12.0	6.0*	100.0	19	14.0–28.0	14.0	100.0
10	5.2–13.2	8.0	153.8	20	18.0–36.0	18.0	100.0
Range		0.8–8.0	6.9–153.8	Range		2.8–29.6	13.7–100.0
Av.	14.0	4.5	50.2	Av.	39.9	15.9	56.7

of R.S. were obtained from 20 individuals on 5 successive days. These subjects were again divided into Groups I and II according to whether their rates of flow of R.S. were originally low or high. Saliva was taken for 15 minutes and the amount calculated per hour (Table II). The average rate for Group I was 14.0 cc./hr. and for Group II 39.9 cc./hr. (Table II, bottom row).

It can be noted that the average fluctuations (Table II, Column B) of Group I were 4.5 as against 15.9 cc./hr. in Group II. The percentage calculation (Column C) however, reveals only a very slight

difference between the two groups (50.2 per cent as against 56.7 per cent). The pronounced differences in fluctuation between Groups I and II over a 2 year period are not found when collections are made on 5 successive days.

For most experiments over a longer period of time it is desirable to reduce the fluctuations of rate of flow to a minimum, which, according to the observations described above, seems to be possible only with a low rate of flow group.

FLUCTUATIONS OF RATE OF FLOW OF RESTING SALIVA DURING 2½ HOURS OF CONTINUOUS COLLECTION

Having found less fluctuation of salivary flow in Group II on 5 successive days than during a period of 2 years the question arose as to the amount of fluctuation during continuous collection. This information was needed to facilitate the interpretation of the results of most investigators, who reported on samples collected over varying periods of time. If, for instance, the rate of salivary secretion was found to vary greatly during a 3 hour period of continuous collection, it might be expected that the salivary composition also would vary during this period. It would become doubtful, therefore, whether the analytical results of such a sample could be compared with the data obtained from another collected over a period of only 10 minutes. Practical experience has taught that in many instances, especially in older individuals, the rate of secretion is extremely slow, in fact sometimes so slow that it is possible to collect only a few drops during an entire hour. This amount is far too small for any routine determination of the composition of saliva, though these individuals, from the standpoint of secretory function and quantitative analysis, are among the most interesting ones.

For the study of fluctuations during continuous collection the rates of flow of 9 individuals were measured (Table III). These were divided as before into Groups I and II. The saliva was collected continuously for 2½ hours in graduated tubes which were changed every 15 minutes, and the individual amounts were recorded. In Table III, Columns A present the range, Columns B, the cc./hr. fluctuations, and Columns C, the percentage fluctuations. The original record, of which a summary only is given in Table III, pre-

sented the interesting finding that the individuals with a lower rate of flow (Group I) showed only slight fluctuations and that the lowest values were obtained within the first 90 minutes of continuous secretion of R.S. After this period the values showed a definite trend upward with the highest ones after one and one-half hours. In Group II such consistent trends were not observed. In some instances the highest rates of flow occurred at the beginning of collection followed by a continuous drop. The average rate for Group I was 12.6 cc./hr. and for Group II 49.1 cc./hr. By comparing the average cc./hr. fluctuations in the bottom rows of Columns B of Table 3 calculated

TABLE III

Rate of flow of resting saliva during 2½ hours continuous collection

GROUP I				GROUP II			
No.	A	B	C	No.	A	B	C
	Range cc./hr.	Fluctuation			Range cc./hr.	Fluctuation	
		cc./hr.	per cent			cc./hr.	per cent
1	3.6– 8.0	4.4	122.2	5	22.3–33.2	10.9	48.9
2	5.6–26.0	20.4	364.3	6	20.7–31.2	10.5	50.7
3	12.4–25.2	12.8	103.2	7	35.6–46.0	10.4	29.2
4	8.4–17.6	9.2	109.5	8	63.2–95.6	32.4	51.3
				9	67.6–84.0	16.4	24.3
Range		4.4–20.4	103.2–364.3	Range		10.4–32.4	24.3–51.3
Av.	12.6	11.7	174.8	Av.	49.1	16.1	40.9

for two and one-half hours collection it is seen that they are less for Group I than for Group II (11.7 as against 16.1 cc./hr.). The average percentage fluctuations (Table III, Columns C), however, give entirely different results. In this case Group I far exceeds Group II (174.8 per cent against 40.9 per cent). This means that the *lower the initial rate of flow of R.S. (Group I) the greater will be the fluctuation during 2½ hours of continuous collection and vice versa.*

Because of these fluctuations which occurred in the main after 60 minutes of continuous collection (especially in the lower rate of flow group), and because the average patient secretes a sufficient amount of saliva for analytical purposes during the first 60 minutes, it appeared

necessary to recalculate the values given in Table III for the first hour of collection. The average rate of flow of Group I was 9.6 cc./hr. and of Group II 45.7 cc./hr.

FLUCTUATIONS OF RATE OF FLOW OF RESTING SALIVA DURING 1 HOUR OF CONTINUOUS COLLECTION

During 1 hour of secretion the average cc./hr. fluctuations (Table IV, Column B) do not differ very much between the 2 groups (4.8 as against 4.2 cc./hr.). The percentage fluctuations (Column C), however, give results similar to those in Table III for 2½ hours of collection; namely, the lower rate of flow group shows far greater fluctuations during the first hour than the higher rate of flow group (64.1 per cent as against 10.3 per cent). This means that the fluctuations of Group I are about 6 times greater than those of Group II for the first hour of collection.

TABLE IV

Rate of flow of resting saliva during 1 hour continuous collection

GROUP	NO.	A	B	C
		Range cc./hr.	Fluctuation	
			cc./hr.	per cent
I	1	3.6– 4.8	1.2	33.3
	2	5.6–11.2	5.6	100.0
	3	12.4–18.8	6.4	51.6
	4	8.4–14.4	6.0	71.4
	Average	9.6	4.8	64.1
II	5	22.3–22.4	0.1	0.4
	6	24.8–31.2	6.4	25.8
	7	38.4–44.2	2.8	7.3
	8	63.2–72.8	9.6	15.2
	9	69.6–71.6	2.0	2.9
	Average	45.7	4.2	10.3

A comparison of the percentage fluctuations during 1 hour with those during 2½ hours of collection indicates a decided increase from 64.1 to 174.8 per cent for Group I and from 10.3 to 40.9 per cent for Group II; this is an approximate increase of 3 to 4 times. If the

relationship of the 2 groups is considered with respect to percentage fluctuation it is found that Group I fluctuates approximately 6 times more than Group II during 1 hour and 4 times more during $2\frac{1}{2}$ hours of collection.

It becomes obvious that if such a difference in the fluctuations of rate of flow exists between a 1 hour and a $2\frac{1}{2}$ hour period of continuous collection, it must necessarily affect the analytical results for calcium and phosphorus expressed in mg. per cent as well as mg./hr. Therefore for the interpretation of findings the total time of collection should be known.

It must be realized that the material in this study permits only preliminary conclusions because of the small number of cases; however, the results give definite leads for future investigations and form a basis for a comparative study with A.S.

The *Summary and Conclusions* as well as *References to Literature* for this and the two succeeding papers have been combined and appear with the last paper on pages 450–456.

HUMAN SALIVA

VIII. A Study of Rate of Flow of Activated Saliva[1]

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Most attempts to correlate salivary composition with oral diseases have been based upon the examination of activated saliva. As yet a correlation has not been found (5, 6). In many instances discrepancies and great variations of results can be traced to the mode of activation. The authors who have used activated samples are of 2 groups: those who activated with such substances as paraffin, small sponges, cotton rolls, lemon drops, etc.; and those who took samples at various times after meals. Variations in the amount and concentration of saliva must be expected if there are differences in the volume, flavor, and consistency of the activator or if the time interval following meals is not standardized.

Changes in composition due to differences in the rate of flow of paraffin activated saliva have been demonstrated by Brown and Klotz (10, 11) and Hubbell and Bunting (15). Brown and Klotz examined salivary samples from 18 students who were divided according to the volume of saliva secreted. Although they found distinct differences in composition between the 3 groups they drew the conclusion for rate of flow "that there is a general constancy of response to paraffin chewing from day to day." Such a relative constancy may exist on 2 days separated by only a short interval, however, it is highly probable that such constancy would not be found if the time interval between sampling were increased and if a larger number of individuals were examined. Furthermore if one compares the ranges of rate of flow for 1 hour of collection of the various groups established by

[1] This research has been carried on under a Fellowship grant from the Carnegie Corporation, New York City.

Brown and Klotz in 1934 and 1937 (Table I, Columns 1, 3 and 5), it can be noted that these ranges differ considerably. According to these authors it would be possible for an individual of the "high producing" group to have a rate of flow of 178.0 cc./hr. on 1 day and 310.5 on another and still to remain in the same class. Such a wide variation in salivary secretion cannot be accepted as indicating a "constancy of response to activation."

The wide gaps between the 3 group ranges for 1 hour samples (Table I) seem to allow a clear classification into distinct groups which appear to be very useful. However these ranges become much larger and overlap considerably when the amounts of saliva collected

TABLE I

*Lowest and highest activated rates of flow found by Brown and Klotz**

GROUP	1934		1937		TOTAL	
	1	2	3	4	5	6
	1 Hour Samples cc./hr.	15 Minute Samples cc./hr.	1 Hour Samples cc./hr.	15 Minute Samples cc./hr.	1 Hour Samples cc./hr.	15 Minute Samples cc./hr.
Low	38.5– 99.0	32.0–120.0	48.0– 83.0	46.0–104.0	38.5– 99.0	32.0–120.0
Medium	132.0–155.5	114.0–170.0	130.0–171.0	100.0–192.0	130.0–171.0	100.0–192.0
High	178.0–310.5	140.0–336.0	203.0–277.0	156.0–328.0	178.0–310.5	140.0–336.0

* Calculated from Brown and Klotz values (10 and 11) and recorded as cubic centimeters per hour.

in each of the 15 minute periods are calculated in cc. per hour and indicate the difficulties encountered with this type of classification. The results of total collection of activated saliva for the 1 hour period do not seem to give a true picture of secretory variations during this hour. Furthermore, it must be surmised that, if great fluctuations occur over four 15 minute periods, finer divisions of the time units would give a still better concept of secretory fluctuations.

FLUCTUATIONS OF RATE OF FLOW OF ACTIVATED SALIVA ON FIVE SUCCESSIVE DAYS

Experimental studies were undertaken to determine the extent of fluctuations of activated salivary flow from day to day. First, on 5 successive days samples of R.S. were collected from 20 individ-

uals then samples of A.S. were procured by the chewing of a 1 gram piece of paraffin 450 times. This procedure has been adopted in this laboratory because some individuals chew very much faster than others and thus in a given time unit vary the amount of activation.

The observations of A.S. secretion are recorded in Table II. Individuals with an originally lower rate of flow of R.S. were again grouped together arbitrarily (Group I) and also those with an originally higher rate of flow of R.S. (Group II). This grouping was chosen because it was found previously (4) that fluctuations of the 2 groups were

TABLE II

Rate of flow of activated saliva on 5 successive days

GROUP I					GROUP II				
	A	B	C	D		A	B	C	D
No.	Range cc./hr.	Fluctuation		Brown & Klotz Classification	No.	Range cc./hr.	Fluctuation		Brown & Klotz Classification
		cc./hr.	%				cc./hr.	%	
1	76.0–85.0	9.0	11.8	L	11	105.0–128.4	23.4	22.3	L, M
2	70.0–101.0	31.0	44.3	L	12	141.3–173.1	31.8	22.5	M
3	171.2–249.7	78.5	45.8	M, H	13	143.0–205.8	62.8	43.9	M, H
4	100.0–121.0	21.0	21.0	L, M	14	199.0–224.4	25.4	12.8	H
5	52.5–69.0	16.5	31.4	L	15	216.0–246.0	30.0	13.9	H
6	120.0–157.5	37.5	31.2	L, M	16	112.0–168.0	56.0	50.0	L, M
7	46.3–67.2	20.9	45.1	L	17	205.7–245.0	39.3	19.1	H
8	135.6–157.3	21.7	16.0	M	18	180.0–230.3	50.3	27.9	M, H
9	63.0–102.0	39.0	61.9	L	19	117.0–174.0	57.0	48.7	L, M
10	65.7–112.5	46.8	71.2	L	20	119.0–225.0	106.0	89.1	L, M, H
Range		9.0–78.5	11.8–71.2		Range		23.4–106.0	12.8–89.1	
Av.	106.4	32.2	38.0		Av.	173.8	48.2	35.0	

distinctly different. The average rate of flow of Group I was 106.4 cc./hr. and of Group II 173.8 cc./hr. (Table II, bottom row).

Columns A and B of Table II indicate that on 5 successive days the fluctuations in cc./hr. are very large for both groups: from 9.0 to 78.5 with an average of 32.2 cc./hr. for Group I, and from 23.4 to 106.0 with an average of 48.2 cc./hr. for Group II. The average is markedly higher for Group II than for Group I which means that the higher the original rate of flow of R.S. in cc./hr. (Group II), the greater is the fluctuation of the rate of flow of A.S. on 5 successive days and vice versa. However, if the percentage fluctuations are calculated

(Table II, Column C) the difference between the 2 groups (Table II, bottom row) disappears almost entirely (38.0 against 35.0 per cent).

If these observations are compared with the groupings of Brown and Klotz, it can be noted in Table II, Column D that on 5 successive days the rate of flow values for each individual do not remain within the range of the original group.[2] The individuals, especially those in Group II, did not show a constancy in response to activation on various days.

It must be noted that the time of collection of the first sample in the experiments of Brown and Klotz was 15 in contrast to an average of 6 to 8 minutes for the individuals in Table II.

FLUCTUATIONS OF ACTIVATED SALIVA DURING 45 MINUTES OF CONTINUOUS COLLECTION

In the light of a previous observation (4) that considerable fluctuations of salivary flow occurred during $2\frac{1}{2}$ hours continuous collection of R.S. for both Groups I and II with maximum rates after 90 minutes it appeared necessary to determine the extent of fluctuations during the continuous collection of A.S. For comparative purposes the same classification into Groups I and II was chosen. Thirty individuals were requested to give R.S. (reported separately (7)) and then 8 successive activated samples (450 chews each). The average total time of collection was 45 minutes.[3] The average rate of flow in Group I was 84.0 cc./hr. and in Group II 86.3 cc./hr. (Table III, Columns A, bottom row).

In comparing the range and fluctuations in cc./hr. and per cent for each individual (Table III, Columns A, B, and C) it can be noted that there is considerable fluctuation in rate of flow during 3,600 chews (8 times 450 chews) although there is very little difference between the 2 groups. If the total figures that have been entered in the bottom

[2] The cc. per hour ranges calculated from Brown and Klotz' Table I (10) for the first 15 minute collection period are:

low producing group: 45.0–120.0 cc. per hour
medium producing group: 120.0–192.0 cc. per hour
high producing group: 180.0–328.0 cc. per hour

[3] The time of collection, amount of saliva and the age and sex of all individuals were recorded but omitted in this report because of limited space.

rows of this table are compared with those of Table II it becomes evident that the percentage fluctuations during continuous collection average almost twice as high as on different days, i.e., 68.8 and 73.9 per cent against 38.0 and 35.0 per cent.

A comparison of these fluctuations with those of Brown and Klotz during continuous activation (Table I, Column 6) reveals that most

TABLE III

Rate of flow of continuously activated saliva

GROUP I*					GROUP II**				
No.	A	B	C	D	No.	A	B	C	D
	Range*** cc./hr.	Fluctuation		Brown & Klotz Classification		Range cc./hr.	Fluctuation		Brown & Klotz Classification
		cc./hr.	%				cc./hr.	%	
1	48.0- 76.3	28.3	59.0	L	16	34.0- 80.0	46.0	135.3	L
2	89.7-119.0	29.3	32.7	L, M	17	36.9- 65.0	28.1	76.2	L
3	48.0- 67.5	19.5	40.6	L	18	15.6- 40.8	25.2	161.5	L
4	49.7- 76.5	26.8	53.9	L	19	38.4- 68.0	29.6	77.1	L
5	45.2- 70.8	25.6	56.6	L	20	60.9-102.0	37.7	58.6	L, M
6	28.8- 55.2	26.4	91.7	L	21	79.0-114.0	35.0	44.3	L, M
7	105.5-145.1	39.6	37.5	L, M, H	22	42.0- 58.9	16.9	40.2	L
8	70.5- 83.0	12.5	17.7	L	23	114.0-175.5	61.5	53.9	L, M, H
9	72.9-112.0	39.1	53.6	L, M	24	106.0-148.0	42.0	39.6	L, M, H
10	48.0-111.0	63.0	131.2	L, M	25	59.3-108.0	48.7	82.1	L, M
11	70.3-149.6	79.3	112.8	L, M, H	26	87.8-152.0	64.2	73.1	L, M, H
12	49.4- 94.8	45.4	91.9	L	27	85.2-122.1	36.9	43.3	L, M
13	90.6-150.0	59.4	65.6	L, M, H	28	104.6-187.0	82.4	78.8	L, M, H
14	74.2-190.1	115.9	156.2	L, M, H	29	103.1-157.0	53.9	52.3	L, M, H
15	130.5-171.0	40.5	31.0	M, H	30	31.2- 60.0	28.8	92.3	L
Range		12.5-115.9	17.7-156.2		Range		16.9-82.4	39.6-161.5	
Av.	84.0	43.4	68.8		Av.	86.3	42.5	73.9	

* Lowest rates of flow of resting saliva of 30 individuals.

** Highest rates of flow of resting saliva of 30 individuals.

*** From eight successive samples, 450 chews each, average time of collection for each sample 6 to 8 minutes.

of these individuals do not remain within the original group, some even falling into all 3 groups (Table III, Columns D). The original complete records showed that the majority (10 out of 15) of individuals of Group I produced the highest amount of A.S. during the first 2 periods of 450 chews showing a slight decrease in the next few successive samples with the lowest amount in the fifth sample. In Group II only 5 out of 15 showed the highest rate of flow in the beginning

while the lowest point was reached in the fourth period of collection. An increase was noted to the eighth sample where the highest value was found (see also *fig. 2* of (7)).

FLUCTUATIONS OF ACTIVATED SALIVA DURING 18 MINUTES OF CONTINUOUS COLLECTION

Activation of the salivary glands produces such an increased quantity of saliva that a very short period of collection, 10 or 15 minutes, will suffice to obtain an adequate amount for analytical purposes. Since the cc./hr. and per cent fluctuations for 45 minutes continuous activation far exceed those observed on 5 successive days a recalculation of the values of Table III was made to demonstrate the fluctuations occurring during the first 3 chewing periods (450 chews each) averaging a total time of $18\frac{3}{4}$ minutes. The fluctuations in Groups I and II for this shorter period of collection were 32.2 and 28.6 cc./hr. and 47.5 and 44.7 per cent which are much smaller than those observed over a period of 45 minutes. They are larger, however, than those observed on 5 successive days (38.0 and 35.0 per cent).

These findings emphasize again the necessity of recording and reporting the rate of flow per time unit and the duration and method of activation as well as the original rate of secretion of R.S. for each individual.

The *Summary and Conclusions* and *References to the Literature* for this and the 2 accompanying papers have been combined and appear with the following paper on pages 450–456.

HUMAN SALIVA

IX. The Effect of Activation on Salivary Flow[1]

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COMPARISON OF THE RATES OF FLOW OF RESTING AND ACTIVATED SALIVA OF 30 INDIVIDUALS

The great variation of results of calcium and phosphorus analyses of activated saliva have suggested that the many stimulating or activating factors render the value of A.S. rather questionable as a medium for comparative purposes. Very little information is available which gives the changes in salivary content produced by chewing a tasteless and inert activator, as for instance paraffin, and still less is known about the individual response to activation of saliva in persons with fast or slow flowing R.S. For this reason 30 individuals were requested to produce R.S. for 15 minutes and then for the production of A.S. to chew a 1 gram piece of paraffin 450 times. The experimental conditions were the same as previously reported (4, 28). The classification into Groups I and II again was made in order to study the influence of differences in rate of flow; Group I included 15 individuals with originally low rates of flow, i.e., up to 20 cc. R.S. per hour, aged 12 to 63 years; and Group II, 15 with over 20 cc./hr., aged 6 to 35 years.

Table I demonstrates the changes in rate of flow from R.S. to A.S. in both groups. Even though the average rate of flow of *R.S.* (Table I, Columns A, bottom row) of Group I is approximately one-fourth that of Group II (9.0 and 39.7 cc./hr., respectively) the ranges for *A.S.* (Columns B) are—surprisingly enough—approximately the same,

[1] Conducted under grants made by the Carnegie Corporation, the California State Dental Association, California Academy of Periodontology, Research Board of the University of California and the Good Teeth Council for Children Inc.

perhaps with a slightly lower average in Group II (103.0 and 89.2 cc./hr., respectively). This seems to indicate that activation produces approximately the same rate of flow of A.S. regardless of whether the original rate of flow of R.S. is low or high. Because of this Group II did not show the same proportional increase due to activation as

TABLE I

Comparison of the rates of flow of resting and activated salivas

GROUP I*					GROUP II**				
		A	B	C			A	B	C
			Paraffin Activated Saliva					Paraffin Activated Saliva	
Number	Age	Resting Saliva cc./hr.	450 Chews cc./hr.	Percentage Change from Resting Value %	Number	Age	Resting Saliva cc./hr.	450 Chews cc./hr.	Percentage Change from Resting Value %
1	13	2.5	76.3	+2,952.0	16	15	21.4	38.0	+77.6
2	25	5.7	112.0	+1,864.9	17	14	22.2	62.2	+180.2
3	12	5.9	66.6	+1,028.8	18	10	25.1	17.0	−32.3
4	14	6.2	54.9	+785.5	19	29	25.2	68.0	+169.8
5	28	6.3	70.8	+1,023.8	20	12	30.0	69.8	+132.7
6	21	6.5	55.2	+749.2	21	22	30.4	85.8	+182.2
7	25	7.0	137.7	+1,867.1	22	15	31.3	52.3	+67.1
8	40	8.8	83.0	+843.2	23	23	32.1	132.0	+311.2
9	21	8.8	112.0	+1,172.7	24	35	38.0	143.0	+276.3
10	29	10.3	111.0	+977.7	25	13	40.2	59.3	+47.5
11	32	10.3	149.6	+1,352.4	26	20	49.8	87.8	+76.3
12	13	11.9	68.7	+477.3	27	23	52.5	122.1	+132.6
13	33	13.5	112.9	+736.3	28	22	65.0	187.0	+187.7
14	31	15.5	190.1	+1,126.4	29	27	66.0	157.0	+137.9
15	63	15.8	145.0	+817.7	30	6	66.6	56.3	−15.5
Range	12–63	2.5–15.8	54.9–190.1	+477.3 to +2,952.0	Range	6–35	24.4–66.6	38.0–187.0	−32.3 to +311.2
Average	26.7	9.0	103.0	+1,185.0	Average	19.1	39.7	89.2	+128.8

* Lowest rates of flow of resting saliva of thirty individuals.
** Highest rates of flow of resting saliva of thirty individuals.

Group I. The pronounced difference between the 2 groups is expressed best in the values calculated for percentage change of rate of flow from R.S. to A.S. (Columns C). An average of +1,185.0 per cent was found in Group I (range: +477.3 to +2,952.0 per cent) in contrast to an average of only +128.8 per cent in Group II (range: −32.3 to +311.2 per cent).

A recalculation of White and Bunting's results (30) based on the

same arbitrary classification into Groups I and II gives findings similar to those presented in Table I with an average percentage change from R.S. to A.S. of +336.8 per cent in the lower rate of flow group and +239.4 per cent in the higher group. This difference is not as marked as found in the 2 groups of this report (Table I) because for our purpose individuals with much lower and higher rates of flow were included also. From these studies, however, it becomes evident that the lower the rate of flow of R.S. the higher will be the percentage increase in flow due to activation and vice versa.

COMPARISON OF CHANGES IN RATE OF FLOW DURING CONTINUOUS ACTIVATION WITH THE ORIGINAL RESTING SALIVA FIGURES

When collection was continued after the initial period of activation and the percentage changes from the R.S. values were calculated

TABLE II

Percentage change of rate of flow from resting to activated saliva

GROUP	NO. OF CASES	AVERAGE RESTING SALIVA RATE OF FLOW cc./hr.	PARAFFIN ACTIVATED SALIVA							
			1	2	3	4	5	6	7	8
			450 Chews %	450 Chews %	450 Chews %	450 Chews %	450 Chews %	450 Chews %	450 Chews %	450 Chews %
I*	15	9.0	+1,185.0	+998.4	+888.2	+843.1	+873.1	+892.9	+887.1	+923.2
II**	15	39.6	+128.8	+107.4	+128.4	+108.4	+134.6	+127.9	+138.3	+144.8

* Lowest rates of flow of resting saliva of 30 individuals.

** Highest rates of flow of resting saliva of 30 individuals.

for a total of 8 successive periods of 450 chews each, the differences between Groups I and II remained very marked. The values for each 450 chews are given in Table II.

Fig. 1 illustrates graphically the figures given in Table II. The great difference between Groups I and II in response to activation is very apparent. Therefore, the lower the original rate of flow of R.S. (Group I) the higher will be the initial percentage increase followed by a slight tendency to decrease in 8 successive samples. This tendency is not found in Group II which has an originally higher rate of flow of R.S.

Fig. 2 presents the actual increase of rate of flow from R.S. to A.S.

expressed in cc./hr. including the values for all 8 periods of activation. The rates of the 16 activated samples fall within a very narrow range with but slight fluctuations regardless of the original rate of flow. Therefore, if A.S. is used for analytical purposes no conclusion can be drawn as to whether the individual has had a slow or fast rate of flow under resting conditions. It becomes apparent that activation

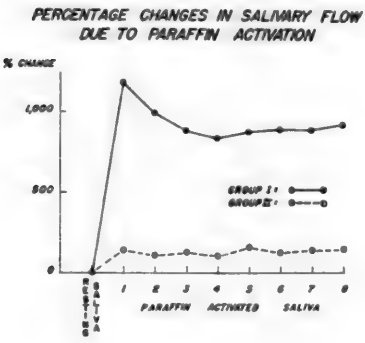

FIG. 1

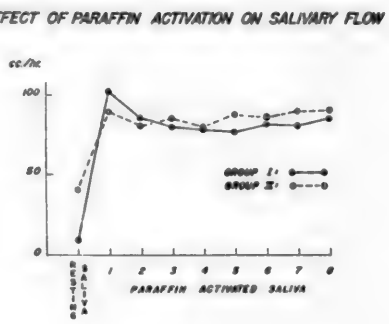

FIG. 2.

disguises any difference between individuals with respect to salivary flow.

SUMMARY AND CONCLUSION

(*Including summaries of papers VII and VIII of this series*)

Samples of resting and of activated saliva have been used indiscriminately by former investigators for purposes of micro-analysis in investigations of possible relationships between salivary composition and oral diseases. The published results contain great discrepancies. These would suggest differences in the types of salivas used as well as differences in the analytical procedures. Since mixed human saliva, in contrast to blood serum, is a typical secretory, and perhaps also an excretory, product it is obvious that fluctuations in rate of flow must cause changes in composition which may provide an explanation for some of the differences in results. With the exception of 1 or 2 investigators no one has paid attention to the influence of rate of flow on salivary composition.

Clinical experience has taught that variations in salivary flow in an individual occur within short periods due to a large number of factors. Little if any information is available, however, with regard to the extent of fluctuations of rate of flow, especially those of R.S. For experimental purposes, it is important to know whether the flow of R.S. of an individual will remain within a narrow range if collected under controlled experimental conditions and after intervals over a period of several years or whether fluctuations occur due to unrecognized factors which would make the interpretation of analytical findings very difficult. It is observed that certain individuals, usually during senility, are unable to produce more than a few drops of R.S. within several hours while others can secrete a sufficient amount for analytical purposes within a few minutes.

When the resting salivary gland is activated, some individuals in spite of the stimulation deliver only very small amounts per time unit, in contrast to others who show a profuse flow. Some individuals, therefore, have in several respects a very concentrated A.S. and others a very diluted one.

The great fluctuations in salivary flow observed clinically made it advisable to study individual rate of flow conditions of R.S. (4) and of A.S. (28). The purpose of these studies has been to gain an insight into the intricate changes that take place when a resting salivary gland is activated by the chewing of paraffin. The investigations furthermore were prompted by the desire to find out which type of saliva, resting or activated, is the most suitable for routine salivary analysis. Comparable age groups were used for this study; and, as the results indicated that the amount of fluctuation depends to a large extent on the original rate of flow of R.S., a classification into two groups was made arbitrarily: Group I including individuals secreting comparatively little R.S. and Group II individuals secreting much R.S. per time unit. Subjects with extreme values were chosen in order to obtain a contrasting picture, and those near the borderline were eliminated temporarily from the series.

The conclusions are summarized as follows (Table III):

1. Over a period of from 1 to 2 years the fluctuations of rate of flow of R.S. (40 individuals) were found to be less in the saliva of persons with lower rates of flow (5.4 cc./hr.) than in those with higher rates

(24.6 cc./hr.). This difference was expressed also in the percentage fluctuations (59.2 per cent and 82.8 per cent, respectively). Therefore, the lower the rate of flow of R.S. the less fluctuation occurs over a period of 1 to 2 years. The greater fluctuations in the group with a high rate of flow may have been due to individually normal variations or to a quicker and greater response to temporary exogenous or endogenous factors.

2. On 5 successive days the cc./hr. fluctuations of rate of flow of

TABLE III

Fluctuations of rate of flow of resting and activated saliva

EXPERIMENT		GROUP	PERCENTAGE FLUCTUATIONS OF RATE OF FLOW					
			Resting Saliva			Paraffin Activated Saliva		
			No. of Cases	Range %	Average %	No. of Cases	Range %	Average %
Different days	Two year period	I	20	13.3–148.4	59.2			
		II	20	26.0–240.9	82.8			
	Five successive days	I	10	6.9–153.8	50.2	10	11.8– 71.2	38.0
		II	10	13.7–100.0	56.7	10	12.8– 89.1	35.0
Continuous collection	2½ hours	I	4	103.2–364.0	174.8			
		II	6	24.3– 51.3	40.9			
	One hour*	I	4	33.3–100.0	64.1	15	17.7–156.2	68.8
		II	6	0.4– 25.8	10.3	15	39.6–161.5	73.9
	18 minutes	I				15	9.0–156.2	47.5
		II				15	11.8–120.6	44.7

* The average collection period for continuously activated saliva was 45 minutes.

R.S. were also less in Group I than in Group II (4.5 and 15.9 cc./hr., respectively) although this difference disappeared in the percentage calculations (50.2 and 56.7 per cent, respectively).

3. During 2½ hours of continuous collection, the cc./hr. fluctuations of R. S. were slightly less in Group I than in Group II (11.7 as against 16.1 cc./hr.). In Group I they were greatest after 90 minutes, showing a tendency for the highest rates to occur toward the end of the entire period. However, the percentage calculations afforded a better

comparison; they indicated that the lower group fluctuates much more (174.8 and 40.9 per cent, respectively). This means that the lower the initial rate of flow of R. S. the greater will be the percentage fluctuation during 2½ hours of continuous collection.

4. During the first 60 minutes of the 2½ hour period of continuous collection the cc./hr. fluctuations of R. S. were found to be approximately the same for Groups I and II (4.8 as against 4.2 cc./hr.), whereas the fluctuations calculated in percentage were 6 times greater in the lower than in the higher group (64.1 as against 10.3 per cent). A comparison of the fluctuations occurring during 2½ hours of collection with those during 1 hour indicated a greater proportional difference between Groups I and II during the shorter period (4 to 1 as against 6 to 1), although the fluctuations in each group were reduced one third to one fourth by shortening the time.

Since such a difference in the fluctuations of rate of flow in these 2 groups exists between a 1 hour and a 2½ hour period of continuous collection the possibility of an effect on analytical results of calcium and phosphorus must be considered. Therefore, for the interpretation of analytical findings the total time of collection must be known.

5. On 5 successive days the response to paraffin activation fluctuated more in Group II than in Group I if results were expressed in cc./hr. (48.2 as against 32.2 cc./hr.); however, in the percentage calculations this difference disappeared (35.0 as against 38.0 per cent).

6. The individual response on 5 successive days showed all degrees of fluctuation from 9.0 to 78.5 cc./hr. in Group I and from 23.4 to 106.0 cc./hr. in Group II. It is therefore hardly possible to speak of a constancy of response to paraffin activation on different days, as stated by Brown and Klotz, because many of the individuals of our series fell under more than one group according to their classification.

7. When A. S. was collected continuously during 8 successive periods of 450 chews each, averaging a total of 45 minutes, considerable variation in rate of flow was found in Groups I and II (28.8 to 190.1 cc./hr. and 15.6 to 187.0 cc./hr., respectively). For practical use this means that if one activates continuously for approximately 45 minutes the response of the salivary glands will vary considerably. The percentage fluctuations for both groups were approximately the same (68.8 and 73.9 per cent, respectively).

8. If the percentage fluctuations of A.S. of Groups I and II on 5 successive days (38.0 and 35.0 per cent, respectively) are compared with those found during 45 minutes of continuous collection of A.S. (68.8 and 73.9 per cent respectively) it can be noted that the fluctuation of the latter series is almost twice as great because of the prolonged collection time.

9. A comparison of the amounts of A.S. of Groups I and II, as obtained during 8 successive collection periods of 450 chews each revealed that the majority of individuals of Group I produced the greatest amount during the first 450 chews; in contrast to Group II which had its lowest values in the first 2 collection periods followed by a slight continuous rise with the highest values in the final period.

10. It becomes apparent from these observations that the salivary glands cannot be "exhausted" with respect to the rate of secretion of both R.S. and A.S. with the possible exception of R.S. in those with high rates of flow.

11. As a sufficient quantity of A.S. for most analytical purposes is obtained in a shorter period of collection than 45 minutes the percentage fluctuations were determined for the first 3 periods of 450 chews each, averaging $18\frac{3}{4}$ minutes. These were found to be greatly reduced, from 68.8 and 73.9 per cent for 45 minutes collection time to 47.5 and 44.7 per cent.

12. A study of the change from R.S. to A.S. indicated that the subjects in Groups I and II reached approximately the same level of A.S. by chewing a 1 gram piece of paraffin, regardless of whether the original R.S. rate of flow was slow or fast, and thereby disguised the original R.S. condition. When the percentage changes from R.S. to A.S. were calculated it was found that the lower the original R.S. rate of flow, the higher was the percentage increase (+1,185.0 per cent in Group I as against +128.8 per cent in Group II). Therefore, although the actual levels of A.S. secretion of these two groups did not differ greatly the difference in percentage increase is very marked (*figs. 1 and 2*).

Activation was accomplished in these experiments by the use of paraffin as a tasteless and inert activator. Changes in volume, consistency and flavor will naturally also change the salivary product, quantitatively and qualitatively.

These studies indicate that the fluctuations of rate of flow of both

R.S. and A.S. depend to a large extent on the duration of collection time, and that prolongation of collection produces even greater fluctuation than that obtained by sampling over a shorter period of time on different days. A comparison of the advantages and disadvantages of the use of R.S. and of A.S. leads to the conclusion that R.S. is better for routine analytical purposes. The deciding factor in this choice is the observation that as a result of activation patients with originally low or high rates of flow of R.S. arrive at approximately the same rate of flow of A.S. thereby disguising the true secretory condition of the resting gland which differs greatly among individuals. For interpretive and comparative purposes therefore, it is necessary to know the collection time as well as the original rate of flow of R.S. for each individual. Furthermore, if more than 1 salivary sample of the same individual is to be used in an experimental study, the percentage rather than cc./hr. fluctuations should be compared. Whether or not the ages of the individuals or seasonal variations influenced the above observations will be treated separately.

The classification into Groups I and II though arbitrary has been very useful in this investigation. However, it is impractical for routine clinical application because of the many borderline cases. This statement applies likewise to the classification developed by Brown and Klotz. The establishment of standards for rate of flow and composition of R.S. and A.S. based on the biometrical evaluation of results of a large number of individuals becomes more and more necessary for all comparative studies.

The foregoing investigations were performed on a comparatively small number of individuals and permit therefore only limited conclusions. However, the observations appear to be most significant for the interpretation of factors which control changes of salivary composition (8, 9).

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A DESCRIPTION OF THE SALIVARY GLANDS OF THE RAT AND A PROCEDURE FOR THEIR EXTIRPATION[1, 2]

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The possibility that the salivary glands have functions in addition to those of aiding deglutition and digestion of food has never been satisfactorily investigated. The only evidence of auxiliary salivary functions consists of occasional reports in the literature claiming increased dental caries (1), effects on blood pressure (2), change in glucose tolerance (3, 4), reduction in dental calcification (5), and variations in the gastric secretions (6, 7, 8, 9) due to absence of the normal salivary secretion. Oral manifestations reported are difficulty in mastication and articulation, dry and atrophic oral mucous membranes, fissured tongue, retention of food debris and, in all cases, the necessity of taking considerable amounts of water with the food.

Using 2 dogs, Fehr (10) in 1862 was one of the first to sense the value of using experimental extirpation of the salivary glands as a means of studying their activities. Because comparative studies on operative physiology (11), type of secretion (12), enzymatic action (13), and histology (14, 15) between the salivary glands of man and the rat have indicated a marked similarity, and because the animal has been so well studied, even in its dental aspects (16, 17, 18, 19, 20), the albino rat (*Mus norvegicus albinus*) was chosen as the experimental animal for the studies of the salivary glands and the development of a technique for their surgical removal.

For convenience and simplicity of presentation, it seems best, first, to give a summary of the nature and anatomical distribution of the

[1] The study upon which this report is based was made possible by funds granted by the Carnegie Corporation of New York.

[2] Presented at the 17th General Meeting of the International Association for Dental Research, Cleveland, March 18–19, 1939 (*J. D. Res.* **18**: 280, 1939).

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glands and their associated structures (from literature and dissections) and, second, to outline the procedure worked out for their extirpation.

THE SALIVARY GLANDS OF THE RAT

There are 4 main groups of salivary glands in the rat: (1) parotid; (2) submaxillary; (3) major sublingual; and (4) minor or lesser sublingual.

Parotid Gland: (*Glandula parotis*). The parotid is the earliest gland to appear (sixth week) in the human and, in most mammals, is the largest of the salivary glands. In the rat it extends from the masseteric region to the clavicle. Its caudal margin rests on the outer half of the cephalic border of the pectoralis major. As the gland lies in its transparent fibrous capsule, a prolongation of the cervical fascia, the fan-shaped diffuse character of its ectal surface can be distinguished. The gland is elongated in the cephalo-caudal direction. It is approximated in the mid-ventral region of the neck by superficial lymph glands and extended over the ventral surface of the submaxillary gland. A triangular mass extends to the ventral and caudal margins of the external ear and into the retroauricular area. "The exorbital lacrimal gland is closely associated with the anterior border and covers the beginning of the parotid duct (Stenson's duct). The duct, formed by the union of three principal branches, crosses the masseter muscle parallel with the buccal and mandibular branches of the facial nerve, and opens opposite the molar teeth," (Greene, 21). It can be traced forward as a fine, translucent, straight structure lying just beneath the fascia over the muscle. The hilum of the gland is several millimeters caudal to the cranial border on the ventral surface and frequently 3 or more ducts are seen to fuse at this point. It continues forward to its termination as a simple duct without associated derivatives and pierces the buccinator muscle. Accessory parotid tissue and small distinct lobules frequently divide the normal gland into 3 or 4 large lobes.

At least 2 major submaxillary lymph nodes are encountered in the substance of the parotid over the ventral surface of the submaxillary gland, (*fig. 3*, C). A series of smaller nodes usually lie embedded deep on the internal surface of the submaxillary ramification. Job (22), in his study of the rodent lymphatic system, mentions that vessels from the tongue and lips lead to these nodes. A medium-sized deep node is often encountered at the branching of the external jugular vein into the anterior facial and transverse veins.

Just beneath the retroauricular ramification, the glistening outline of the facial nerve (seventh cranial nerve) can be discerned in the glandular tissue. Several small branches are given off before entrance into the parotid, but as in the human (23), the nerve divides into its 2 main divisions, the tempero-facial and cervico-facial, before emerging from the gland. These divisions supply the nose, skin, buccinator, and orbicularis oculi and orbicularis oris muscles. Small twigs of the facial pierce the parotid. The cervical division is most easily localized by tracing back from the marginal mandibular nerve. Parotid branches from the auriculo-temperal branch of the fifth cranial also enter this auricular ramification. Fibers from the great auricular nerve derived from the cervical and brachial plexuses, emerge from the shoulder region and penetrate the parotid. All branches, except the division which supplies the ear, are severed in removal of the gland. It is not necessary to maintain intact the fibres from the carotid plexus found in this region.

The blood supply to all the salivary glands is very rich. The parotid gland covers the posterior auricular artery which, in its course to the auricle and adjacent structures in the occipital region sends branches into the substance of the parotid. Terminal arterioles from the superior cervical artery supply the parotid on its internal surface as the artery courses superficially in this region. It is from this artery that the submaxillary nodes receive their blood supply. Occasional twigs coming directly from the external carotid supply the parotid.

Below the mandible the anterior facial vein receives 4 or 5 small glandular branches from the lymph nodes of the submaxillary region and parotid gland. Similarly, tributaries of the posterior facial vein pick up venules from these structures. These afford the main portals of drainage for the parotid gland and submaxillary lymph nodes. All of these structures are encountered on the internal surface of the parotid.

When the clavicular and auditory lobes of the gland are freed, the submaxillary portion will be found to lie in close proximity to the large submaxillary lymph nodes mentioned above. By tracing the blood vessels of the region the smaller accessory nodes can most conveniently be located.

Submaxillary and Major Sublingual Glands: (*Glandula submaxillaries and Glandula sublingualis major*). The submaxillary and major sublingual glands of the Rodentia were distinguished as independent structures by Loewenthal in 1894 (14). In the adult rat the 2 glands present grossly a unilobular apprearance (*Fig. 4*). The outline may be distinguished by their dark color and bulging, almond-shaped contour, lodged in the infrahyoid region near the median line and ex-

tending nearly to the manubrium. Those of opposite sides are separated only by a narrow connective tissue septum. Molded anteroventrally over each pair of glands are the large submaxillary lymph nodes.

The major sublingual lies close to the latero-anterior surface of the larger submaxillary near its foremost part, but is distinctly outlined after the common capsule is removed. It is roughly rectangular in shape and fits into a depression in the large gland. This gland is frequently absent in the human. In the rat, however, the major sublingual gland is well formed, and as pointed out by Chievitz in 1885 (24), it is not an hypertrophied Rivinian gland. Comparative anatomists now designate it as the Bartholinian element which drains by the duct of Bartholin. Carmalt's (25) observation that the submaxillary (Wharton's) duct and greater sublingual ducts are given off near the cephalic poles and course forward beneath the mylo-hyoid to open by independent parafrenular openings on the plica sublingualis, has been verified in this laboratory by several cross-section studies of the head. The orifice of the major sublingual is usually close to that of the submaxillary or slightly caudal to it, but in some few instances has been found to open into the submaxillary duct.

The nerve supply to the glands is derived from the submaxillary ganglion, and from branches of the lingual, chorda tympani and sympathetics, all of which enter with the blood vessels at the hila. Glandular branches of the external maxillary artery furnish the blood supply. A large division turns posteriorly and supplies the submaxillary and major sublingual glands, entering in company with the ducts. A second branch offers added blood supply to lymph glands in this region. The hyoid tributary of the anterior facial vein is joined in the mass of supporting tissue at the hilum of the submaxillary by a glandular branch of the submaxillary and major sublingual glands.

Minor Sublingual Glands: (*Glandulae sublinguales minores*). The minor sublingual glands are the smallest of the salivary glands found in the rat. They are true simple glands, and do not possess a distinct capsule as do the larger compound glands. In location, they correspond to the congregated heterogeneous elements in the human known as the sublingual glands. Anatomists consider them Rivinian structures or, more commonly, the alveo-lingual mass, and it is probable

that they do not represent as highly organized structures as do the greater glands. As in the human, the numerous ducts of Rivinus sometimes join the submaxillary duct; most of them however open separately into the floor of the mouth on either side of the frenulum linguae (plica sublingualis). A network of arterioles and venules from adjacent vessels affords the blood supply.

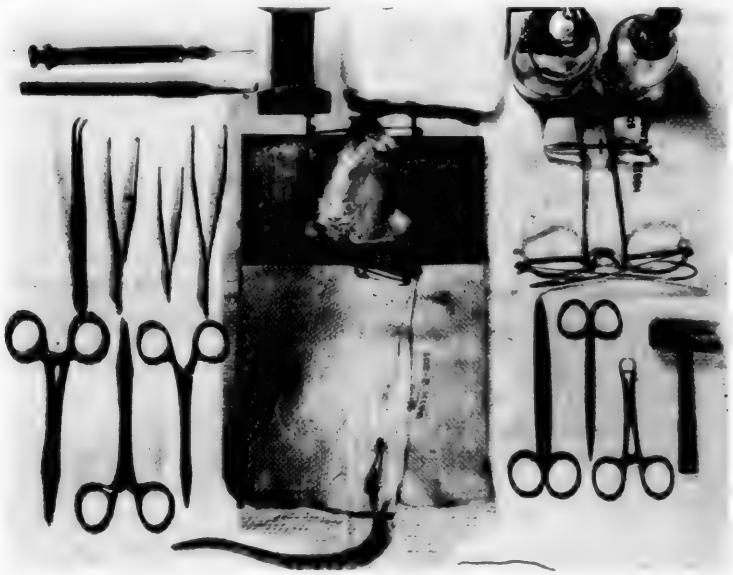

FIG. 1. Operating board, instruments, and materials used in operation

TECHNIQUE OF REMOVAL

It has been found best to remove the salivary glands in 3 stages: first stage, is the removal of the parotid, submaxillary, and major sublingual on one side; second stage, when healing is complete the process is repeated on the other; third stage is the bilateral cauterization of the 2 minor sublingual glands.

The technique to be described embodies ordinary aseptic procedures. Instruments and materials are used which best suit the convenience of the operator. We have employed several small eye instruments which have proven very satisfactory (*fig. 1*). Some type of fastening board which will assure immobility of the rat in the dorsal position is necessary. Intraperitoneal injections of sodium pentobarbital (Nembutal) are given for anesthesia. A stock solution of 1 grain to each cubic centimeter is diluted with saline 1 to 5 for injection and sterilized before use. Administration of 40 mgm. per kg. of rat will produce anesthesia with a maximum duration of 2 to 3 hours. After injection it is best to wait at least 10 minutes before operating.

The use of binocular magnifying glasses or a binocular microscope of about 15 diameters magnification is necessary for the dissection of smaller structures.

Stage I: For the unilateral removal of parotid, submaxillary and major sublingual, the incision is oriented by palpating the lower border of the hyoid bone and (caudally) by the manubrium. An imaginary line is drawn between these 2 points. Laterally, approximately half-way between this mid-ventral line and the ear an incision is made. The cut should not include the richly vascularized deeper layers of subcutaneous tissue at first. This tissue is carefully separated by blunt dissection avoiding the larger arteries.

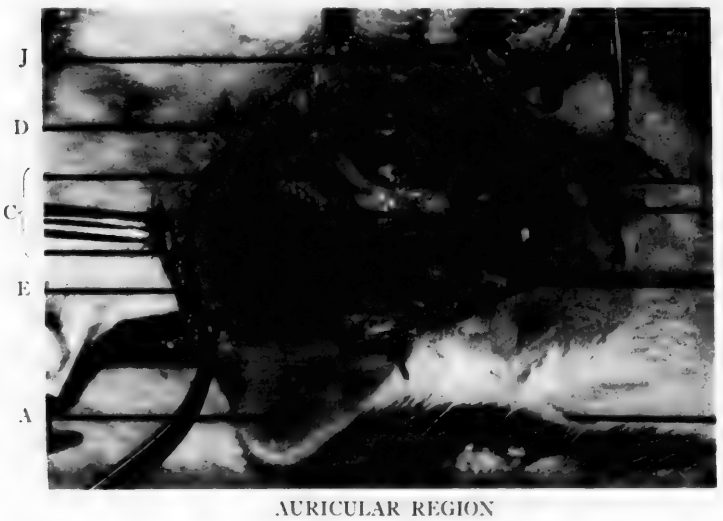

FIG. 2. Exposure of parotid; submaxillary, and major sublingual glands after retraction of skin and subcutaneous tissue. A, ear; B, eye; C, diffuse parotid gland; D, submaxillary and major sublingual glands appearing as one structure; E, exorbital lacrimal gland; F, G, submaxillary lymph nodes; H, marginal mandibular nerve (freed); I, parotid duct; J, blood vessels of subcutaneous tissue. Ducts of submaxillary and major sublingual glands not visible. (Incision extended for photographic purposes.) Approximately 2.8 ×.

Such a precaution minimizes the post-operative sloughing so often encountered in the region of the incision. With further dissection all structures of the common integument are separated from underlying tissues medially to the mid-line and antero-laterally to the posterior limit of the exorbital lacrimal gland. The surface of the parotid must be freed where it diffuses in minute terminations in the region of the posterior limit of the ear. The area is then opened down over the region of the clavicle (*fig. 2*).

In the *removal of the parotid gland* it is well to begin at the tip of the retroauriuular ramification. The gland is gently freed from its delicate sheath of connective tissue at this point and lifted forward. The temporo-facial and cervico-facial branches of the facial

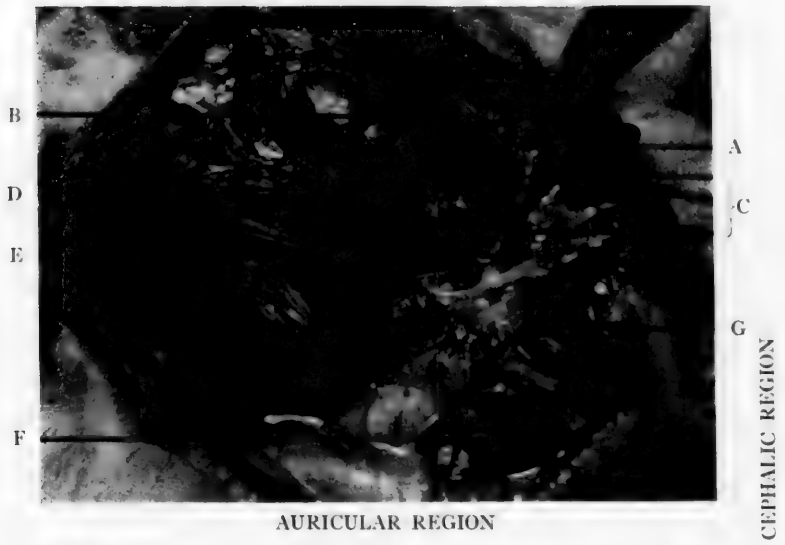

FIG. 3. Medial retraction of dissected right parotid gland. A, parotid; B, outline of submaxillary and major sublingual glands; C, submaxillary lymph nodes; D, accessory-lymph node; E, blood vessels; F, exorbital lacrimal gland; G, ligature in place to tie off parotid. Approximately 4.3 ×.

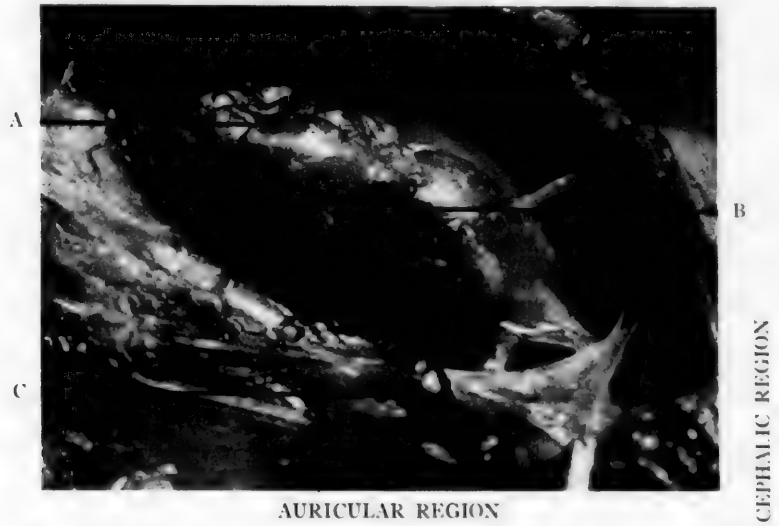

FIG. 4. View of the submaxillary and major sublingual glands in position after adjacent overlying structures have been removed. A, submaxillary gland, B, major sublingual gland; C, submaxillary lymph node. Note close proximity of two salivary glands. Approximately 5.7 ×.

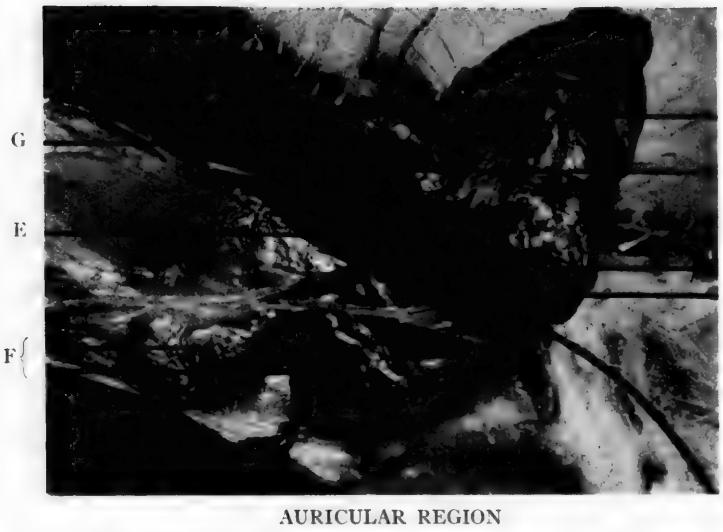

FIG. 5. Retraction of submaxillary and major sublingual glands preparatory to ligation and excision. A, submaxillary gland; B, major sublingual gland dissected free; C, duct of submaxillary; D, duct of major sublingual; E, branch of external maxillary artery supplying glands; F, submaxillary lymph nodes; G, ligature. Approximately 5.7 ×.

FIG. 6. Unilateral view of area after completion of first stage extirpation of glands. Note remaining submaxillary lymph nodes, A; B, exorbital lacrimal gland. Approximately 3.5 ×.

nerve must be dissected out as they supply structures in the anterior portion of the head region. The parotid branches of the auriculo-temporal entering this ramification can be severed. The auricular branch of the great auricular nerve must be maintained intact. Fibres from the carotid plexus are routinely severed. Parotid arterioles of the posterior auricular artery can be severed during the removal. Since the submaxillary lymph nodes are supplied by the superior cervical artery it is well to dissect the branches sufficiently to allow the blood supply of these nodes to remain intact. Only those venules of the posterior facial vein which carry off the blood from the parotid should be destroyed. When the entire parotid structure is free it is retracted mesially and the duct, regional ar teries, and veins at the hilum are all tied off with 1 ligature (*fig. 3*). Numerous small vessels encountered previously can usually be severed without ligation or hemorrhage.

Picking up the tail of the *submaxillary salivary gland* (*fig. 4*), the heavy fibrous capsule is stripped off until the ducts, arteries, and veins are encountered at the hilum. The small *major sublingual gland* will adhere tightly to the submaxillary gland and will be removed at the same time. The structures at the hila, then, are ligated with 1 ligature and both the glands freed (*fig. 5*). This completes the first stage of the operation (*fig. 6*) and the incision is closed with a mattress skin suture.

Stage 2: The first stage of the operation is repeated on the other side, after healing is complete, and the animal has again returned to normal weight.

Stage 3: This consists of the *removal of the minor sublingual glands.* With a wire-frame attachment placed on the operating board the mouth is opened and an oral incision is made in the posterior region of the alveolingual sulcus. The oral mucous membrane is pushed laterally to enlarge the opening and the base of the area is seared by means of a small platinum electric cautery. In this way, small remaining independent masses of glandular tissue in the region will either be destroyed or have their outlets scarred.

DISCUSSION

Although marked differences in the size of glands from opposite sides of the head are normally encountered, weight measurements and histological study have shown that compensatory hypertrophy occurs on the unoperated side within a few weeks after the glands from 1 side have been extirpated. This results grossly in an increase in bulk and weight of the submaxillaries over normals as computed by Hatai (26) and the more distinct glands produced in this way facilitates operation on the second side.

In general, the animals operated upon have withstood the procedure well and from all appearances remain in good health. Weight losses have occurred after operation, probably through a temporary interference with nutrition, but by occasionally moistening the food with water this has been reduced to a minimum, seldom amounting to more than 10 per cent deviation from normals compared either to controls from the same litter or standards by Donaldson (27).

The prevalence of dental decay and the interest of the profession in

its solution would suggest one of the first and most profitable problems applicable to the technic outlined. T. J. Hill (28) has summarized his own findings and admirably reviewed the investigations of others regarding the influence of saliva upon the growth of bacteria in the mouth. Whether it is the group favoring the chemico-parasitic theory of W. D. Miller (29, 30, 31, 32) or those interested in the influence of systemic factors on the structure of the tooth (33, 34, 35, 36), either school is cognizant of the influence salivary secretions have on the susceptibility or immunity to dental caries. In addition, the work of Bibby, Hine and Clough (37) on the anti-bacterial potency of saliva serves to demonstrate the value of the salivary function in regulation of the oral flora.

Mucilagenous bacterial material, precipitated from mucous saliva, forms what is known as the dental plaque and is held by many to be the prime offender in the process of dissolution of enamel and dentin (38, 39). Day (40) believes an increase in ptyalin activity from serous glands is efficacious in the reduction of caries. By selective extirpation, a partial xerostomia could be obtained which would be either almost wholly mucous or serous in character as desired, depending upon the glands allowed to remain.

The submaxillary salivary gland is a mixed gland, and could not be retained in either case. Extirpation of the parotid (serous) would produce a mucous saliva. On the other hand, removal of the major sublingual gland (mucous), and the small minor sublingual glands, would leave a saliva predominantly serous in character, except for the minor secretions of the small mucous glands in the membranes of the mouth.

SUMMARY

1. An anatomical description of the salivary glands of the rat is presented. 2. A 3 stage technique devised for the complete or partial extirpation of the major salivary rat glands is described. Developmental and functional analogies are mentioned in relation to the human salivary glands. 3. It is suggested this method offers a valuable means for study of function of the salivary glands both in their relation to the metabolic activities of the body and in relation to oral disease.

The author is pleased to acknowledge here the assistance given by Dr. James H. Wills, Jr. in operative procedures, Dr. Martin Deakins in photography, Dr. George W. Corner in correction of anatomical details, and Dr. Basil G. Bibby for many valuable suggestions during the course of the work.

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